Automated Morphometric Analysis of Mammospheres: Characterization of Breast Cancer Drugs

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Abstract

Mammospheres are commonly used in cancer research to study cancer pathogenesis[1] and to identify new therapeutic agents[2]. Counting and morphometric analysis of suspension sphere cultures, such as mammospheres, are frequently done manually and thus not amenable to high throughput applications. The Colony Counting application on the Celigo® cytometer provides accurate and consistent morphometric analysis of mammosphere populations in a non-destructive manner. The system records whole well images of multi-well (6-wells to 1536-wells) plates and allows for correlation of mammosphere morphology with anti-cancer drug properties. A panel of cytotoxic drugs, including doxorubicin and paclitaxel were used to study their effects on various breast cancer cell lines such as MDA-MD-436, MCF-7, SKBR3 and MDA-MB-231. Results show that the Colony Counting application can also be used to evaluate the clonogenicity and self-renewal of cancer stem/tumor-initiating cells by automatically analyzing mammosphere populations. The Colony Counting application on Celigo provides an efficient, reproducible and automated method for assessing the number, size, and morphology of cancer spheroids within multiwell plates.

Experimental Design

- Mammospheres were formed via trypsin dissociation of adherent parental cultures (Parental A) or spheroid parental cultures (Parental S), plated in low attachment surface plates and analyzed repeatedly over a 16 day period by brightfield imaging.
- Mammosphere formation in the presence of doxorubicin[3], paclitaxel, 8-quinolinol and salinomycin was studied over a two week period.
- End point viability was assessed using calcein AM, and propidium Iodide.

Brightfield Images

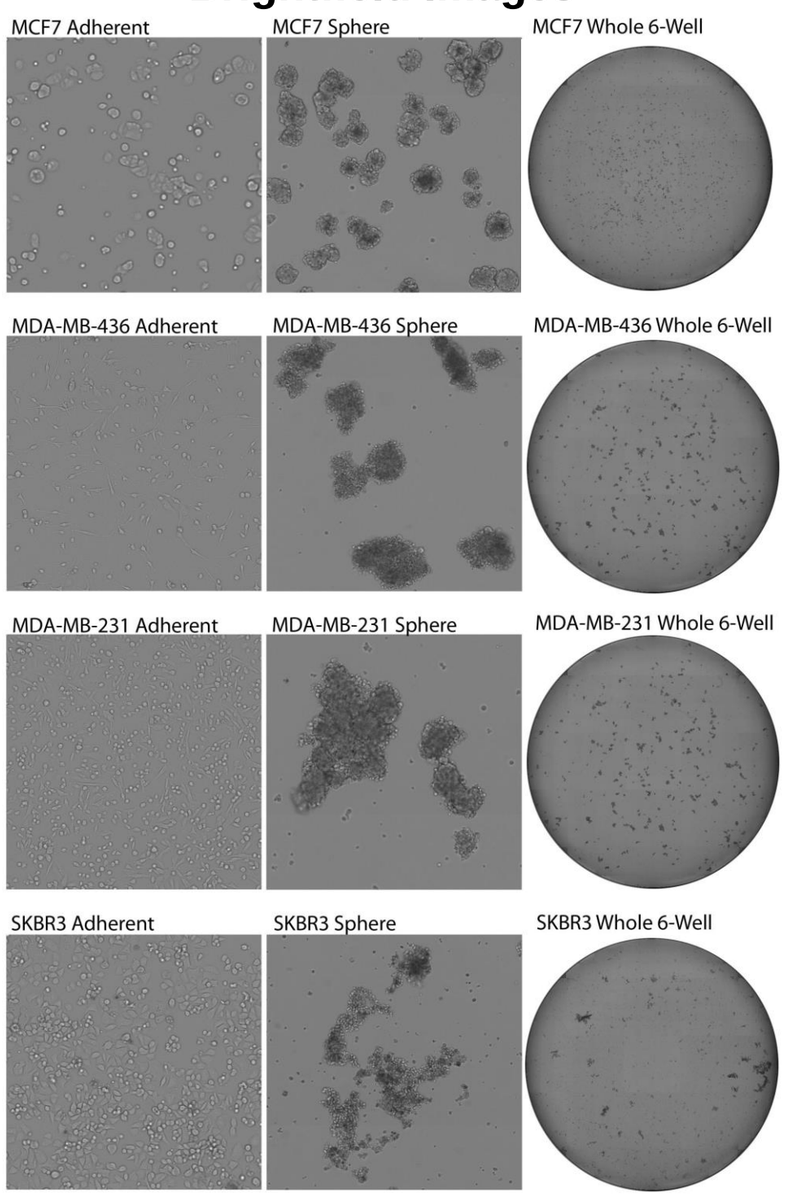

Figure 1. Breast cell cancer lines (MCF7, MDA-MB-436, MDA-MB-231, SKBR3) were grown adherently in 96-well TC-treated plates (left column) and spherically in 6-well low attachment plates (middle zoomed-in & right column).

Sphere Segmentation

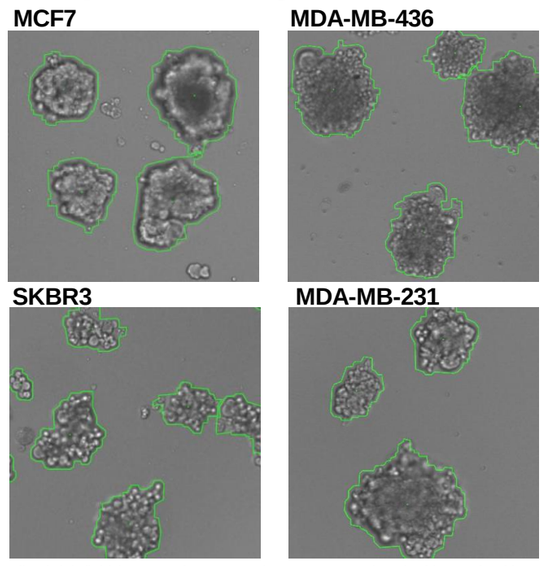

Figure 2. Celigo brightfield images of mammospheres were analyzed with an algorithm that defines and outlines the spheres.

- The Celigo software accurately identifies and segments multicellular objects, such as mammospheres, and automatically provides a series of morphometric measurements, such as sphere area and long axis.

Automated Morphometric Analysis

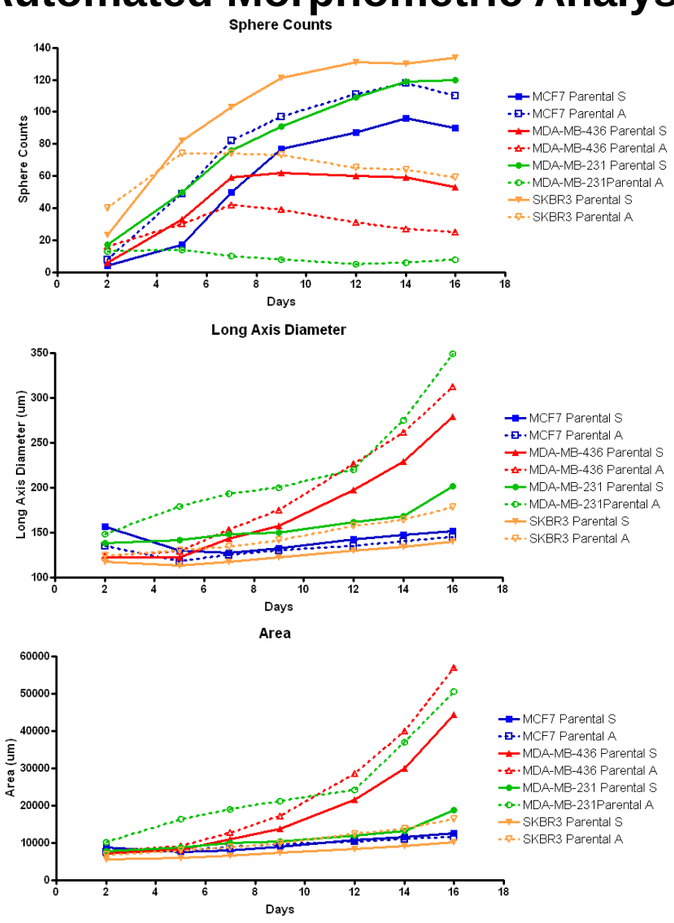

Figure 3. The Colony Counting application was used to quantify mammosphere formation and growth. Four cell lines originating form adherently grown cultures (Parental A) or spherically grown cultures (Parental S) were grown spherically over 16 days. Counts, long axis diameter, and area automatically reported by the software were plotted over time.

- Mammospheres originating from adherent or spheroid parental cultures exhibited different growth rates.
- When originating from adherent parental, MDA-MB-231 and MDA-MB-436 formed large mammospheres while MCF7 and SKBR3 formed smaller mammospheres.
- MDA-MB-231, MDA-MB-436 and SKBR3 mammo-spheres originating from adherent cultures grew in size rather than in total number of spheres.
- Rapid whole well imaging (5-20 minutes/plate) and automated image analysis greatly facilitates longitudinal studies on mammosphere formation and growth.

Hoechst Efflux

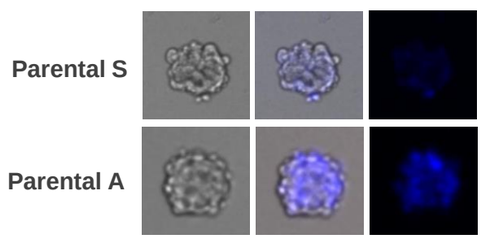

Figure 4. Hoechst stained MCF-7 Mammospheres

- MCF7 spheres generated from parental spheroid cultures exhibited Hoechst efflux whereas spheres originating from parental adherent cultures did not[4].

Sphere Formation *vs*. Compound Treatment

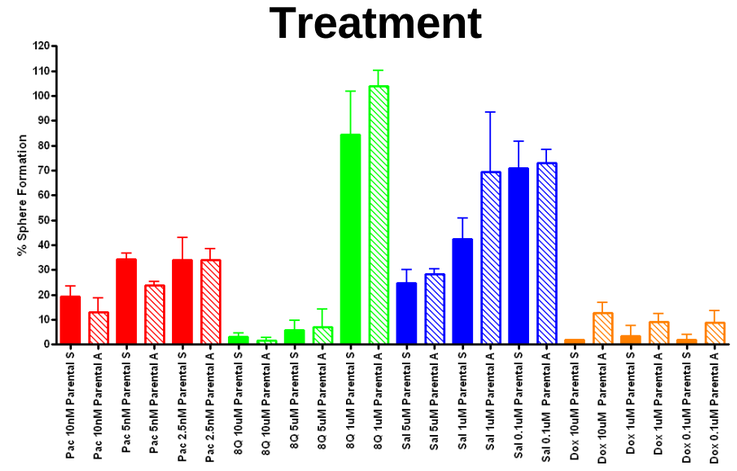

Figure 5. Percentage counts from Day 7 of drug treatment of MCF7 spheres. Celigo brightfield images of mammospheres were analyzed for sphere counts. Sphere counts were normalized to untreated controls for each parental type (S - spheroid and A – adherent). Pac = paclitaxel; 8Q = 8-quinolinol; Sal = salinomycin; Dox = doxorubicin. n=3, error bars indicate standard deviation.

- Sphere formation was inhibited by all drugs tested.
- Sphere formation of cells exhibited differential sensitivity to compound treatment depending on the source of the parental cells, i.e. from spheroid or adherent cultures..

End Point Viability

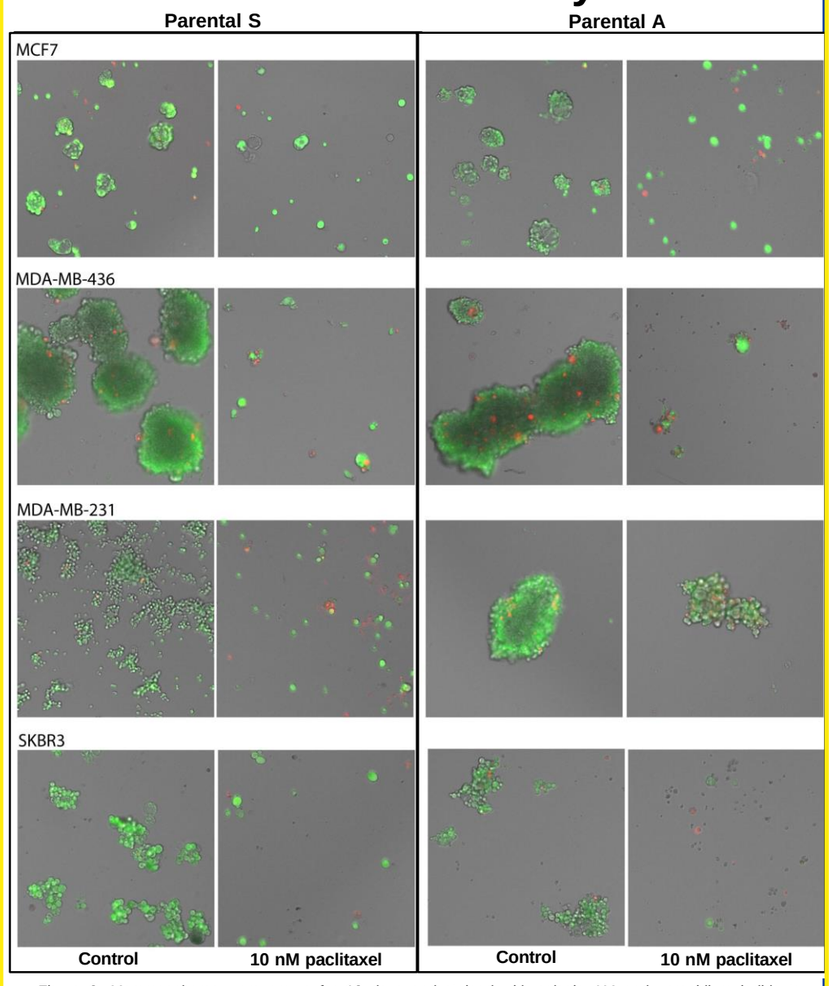

Figure 6. Mammospheres were grown for 16 days and stained with calcein AM and propidium iodide to determine cell viability and sphere formation. Control Parental S spheres vs. 10 nM Paclitaxel treated (left two columns). Control Parental A spheres vs. 10 nM paclitaxel treated (right two columns).

- Viability and sphere analysis showed that paclitaxel strongly inhibited sphere formation without inducing significant cell death in the various breast cancer cell lines.

Conclusion

The Celigo adherent cell cytometer is a bench top imager suitable for a variety of cancer related applications. We have shown the utility of Celigo's rapid whole-well brightfield and fluorescent imaging, coupled with automated morphometric analysis to quantify compound effects on mammosphere formation and growth.

References

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3. Howell, S.J., et al., Prolactin receptor antagonism reduces the clonogenic capacity of breast cancer cells and potentiates doxorubicin and paclitaxel cytotoxicity. Breast Cancer Research (2008) 10:R68
4. Naylor, C.S., et al., side population/ABCG2-positive cells represent a heterogeneous group of hematopoietic cells: implication for the use of adult stem cells in transplantation of plasticity protocols. Bone Marrow Transplantation (2005) 35:353-360