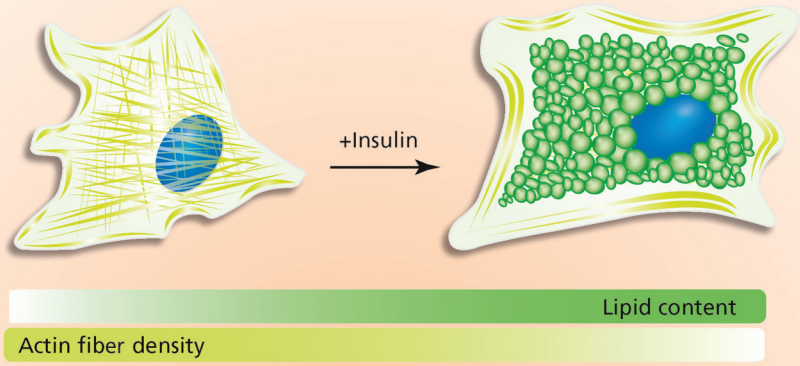

APPLICATION NOTE

Cellular Imaging and Analysis

Key Features

- Adipogenic differentiation of human mesenchymal stem cells (hMSCs)
- Classification of cells based on differentiation markers
- Texture analysis of cytoskeletal rearrangement

Analysis of Stem Cell Differentiation using the Operetta High Content Imaging System

Background

Human mesenchymal stem cells (hMSCs) are multipotent cells present in the bone marrow that can replicate as undifferentiated cells. They have the potential to differentiate to lineages of mesenchymal tissues such as bone, cartilage, muscle and fat. When cultured *in vitro*, these cells can undergo osteogenic, chondrogenic or adipogenic differentiation depending on the culture conditions [Pittenger *et al.,* 1999]. Consequently, it has been suggested that hMSCs can be used for tissue engineering [Caplan, 2007]. To this end, the search for compounds that drive hMSCs to a distinct lineage decision is of great importance.

In this study, the Operetta® High Content Imaging System was used to study the cellular changes associated with adipogenic differentiation of hMSCs. The accumulation of intracellular lipid droplets was measured in order to classify cells that had already differentiated into adipocytes [Pittenger *et al.,* 1999]. Moreover, the high content imaging approach allowed for the study of changes in the actin cytoskeleton which have been associated with the progressing differentiation of hMSCs [McBeath *et al.,* 2004; Treiser *et al.,* 2010].

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Application

To study adipogenic differentiation, human adipose-derived stems cells (ADSCs) were used that have functional and phenotypic characteristics very similar to those of bone marrow-derived mesenchymal stem cells (StemPro®, Invitrogen™, R7788-110). Cells were seeded at a density of 500 cells per well into 384-well CellCarrier™ microtiter plates (PerkinElmer, 6007550). To stimulate differentiation, cells were cultured in serum-supplemented DMEM with the addition of dexamethasone, IBMX, indomethacin and different concentrations of insulin (1.74 µM or 10 µM). Non-differentiating control cells were cultured in MesenPro RS™ Growth media (Invitrogen™, R7780-110).

After 14 days of differentiation, cells were fixed with 4% formaldehyde and permeabilized with 0.1% Triton™-X-100. Subsequently, cells were stained with a dye cocktail containing 10 µg/mL DAPI (Sigma-Aldrich®, D8417), 5 µg/mL Phalloidin-TRITC (Sigma-Aldrich®, D1951) and 1:200 LipidTOX™ Green (Invitrogen™, 34475). Images were acquired on an Operetta System using a 20X high NA objective in wide-field mode. Differentiated cells showed increased lipid accumulation in response to increasing insulin concentration. In addition, an altered actin cytoskeleton pattern was visible in insulin-treated cells (Figure 1).

For quantitative analyses, individual cells were segmented based on the DAPI nuclear stain using the Find Nuclei building block in the Harmony® High Content Imaging and Analysis Software. Using the Select Cell Region building block, a ring region of 15 px width was then created around the individual nuclei (Figure 3A) and the mean intensity of the LipidTOX™ Green staining in this expanded region was used to classify cells into adipocytes and non-adipocytes. The level of adipocytes increased to 29% and 49% with 1.74 µM and 10 µM insulin respectively (Figure 2).

With increasing insulin concentration, a reorganization of actin was observed into thicker fibers that localize to more distal cell regions. To quantify this phenotype, texture analysis was performed on the TRITC image using the Harmony software and the 'SER Features' method. The 'SER Features' method analyzes the occurrence of typical patterns in the intensity structure of an image. The 'Valley' pattern was selected, at 1 px width to search for small interspaces between actin fibers in the 15 px ring region around the nucleus (Figure 3A). An increase in the calculated SER Valley measurement was observed with increasing insulin concentration, reflecting the loss of thin actin fibers from the expanded nuclear region (Figure 3B).

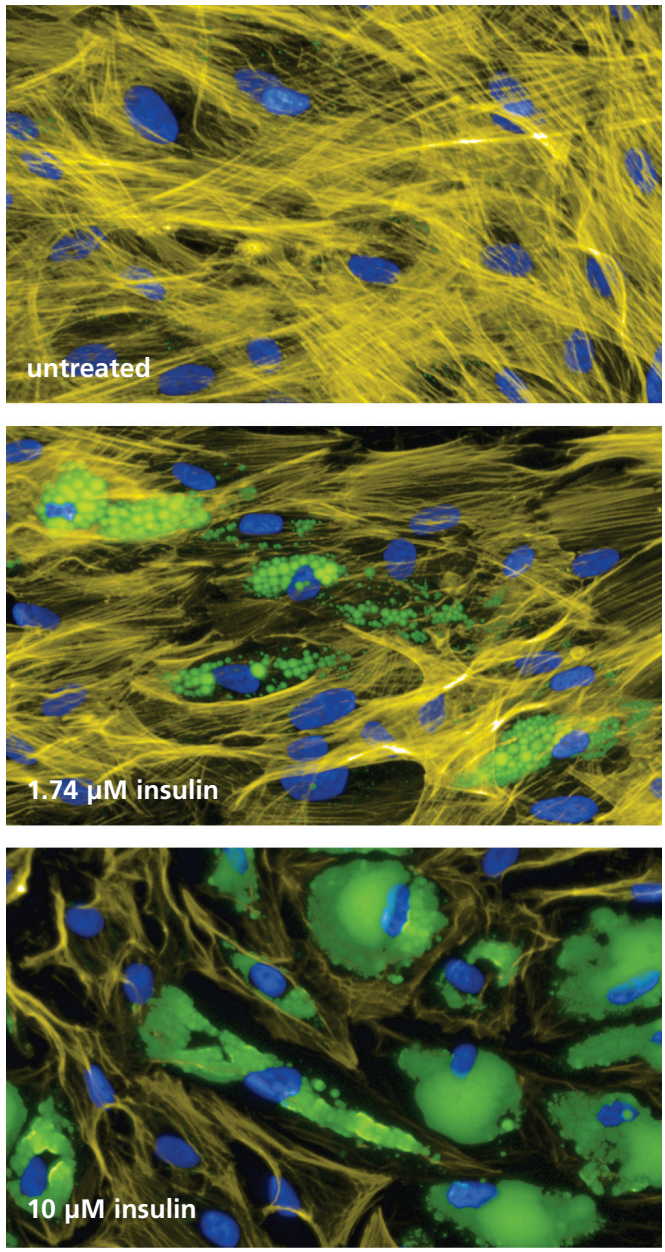

Figure 1. Example images showing differentiating hMSCs and control cells with stained actin cytoskeleton (yellow), lipid droplets (green) and nuclei (blue). Upon differentiation, an increase in the cellular lipid content is visible. The actin cytoskeleton also changes from very thin fibers covering the entire cell to thicker structures that locate more to the cell perimeter.

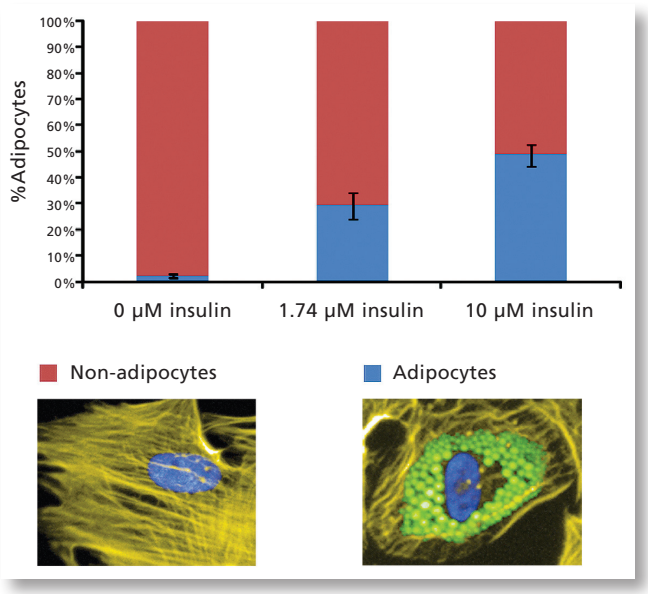

Figure 2. The percentage of adipocytes increases with higher insulin concentrations. Cells were classified based on the mean intensity of LipidTOX™ Green staining in an expanded ring region around the nucleus.

Conclusion

We here present a high content imaging application to study different aspects of stem cell differentiation in a quantitative way. We were able to robustly distinguish between differentiated and undifferentiated cells using LipidTOX™ Green as a differentiation marker for adipocytes. The texture analysis module of the Harmony software allowed additional quantification of rearrangements in the actin cytoskeleton. These sophisticated software capabilities allowed for the key advantages of imaging applications over fluorescence activated cell sorting (FACS) to be fully exploited, and have already been used to predict the lineage decision of hMSCs differentiation at early time points [Treiser *et al.*, 2010]. Consequently, high content imaging based assays have been shown to be the ideal method for accelerating compound screening of hMSCs differentiation in the future.

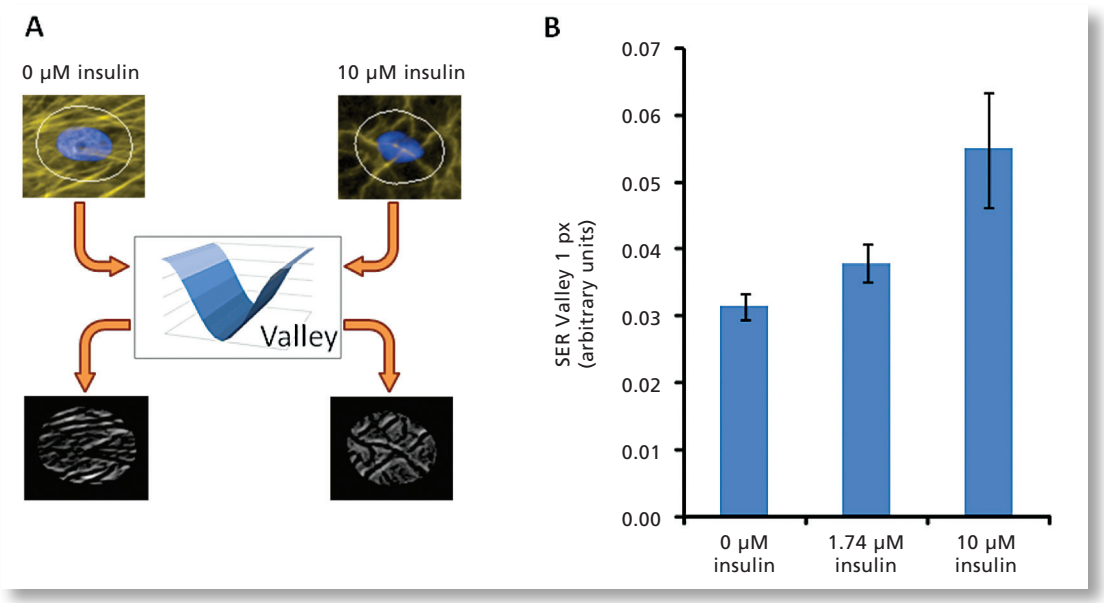

Figure 3. (A) Texture analysis within the Harmony Software using the 'SER Features' method. Here, the SER 'Valley' was used with a scale of 1 px. The lower images show in gray the valleys found in the defined region (shown by the white circles in the upper images). (B) In insulin treated hMSCs, more valley-like patterns occur as the actin fibers localize more to the cell borders.

References

Pittenger, M.F., Mackay, A.M., Beck, S.C., Jaiswal R.K., Douglas R., Mosca, J.D., Moorman, M.A., Simonetti, D.W., Stewart, C. and Marshak, D.R. (1999): Multilineage potential of adult human mesenchymal stem cells. *Science*, 284 (5411), 143-147.

Caplan, A.I. (2007): Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J. Cell Physiol.,* 213 (2), 341-347.

McBeath, R., Pirone, D.M., Nelson, C.M., Bhadriraju, K. and Chen, C.S. (2004): Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell,* 6 (4), 483-495.

Treiser, M.D., Yang, E.H., Gordonov, S., Cohen, D.M, Androulakis, I.P., Kohn, J., Chen, C.S. and Moghe, P.V. (2010): Cytoskeleton-based forecasting of stem cell lineage fates. *Proc. Natl. Acad. Sci. USA,* 107 (2), 610-615.

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