High Content Imaging Based Mapping of Stem Cell Phenotypes

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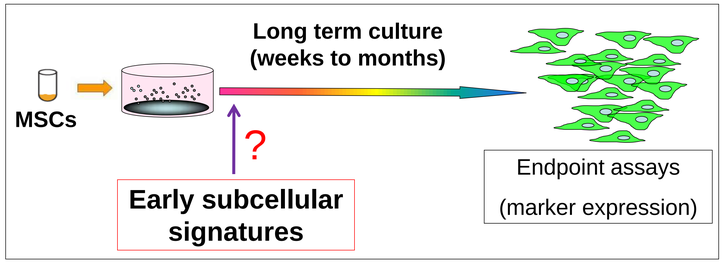
Motivation and Background

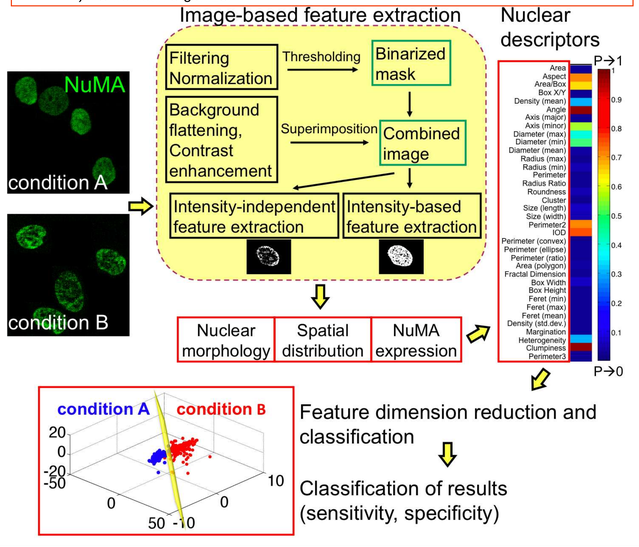
Stem cell phenotype commitment (i.e. differentiation, oncogenic transformation) are processes that take several weeks to fully develop and conventionally are assayed via long-term phenotypic marker expression. There are many factors that contribute to the modulation of early gene expression that leads to lineage commitment, such as cell morphology and transcription factor expression. We report that early variations in nuclear subcellular morphologies could provide extra insight in predicting the phenotype commitment of different cell lines. Our novel approach captures a large number of quantitative features (termed descriptors) that define the "characteristic" state of cells from nuclear mitotic apparatus (NuMA), a nuclear protein reporter. The large pool of nuclear descriptors are then merged into a "composite representation" feature space through dimensionality reduction, data fusion, and classification techniques. Using this methodology, we have graphically shown that mesenchymal stem cells (MSCs) differentiation into osteogenic lineage could be captured as early as 72h, as denoted by our NuMA based nuclear descriptors. We have also been able to capture early cancerous transformation of MSCs. To show the robustness of this methodology, we have also looked at identifying embryonic stem (ES) cells lineage commitment as well as oligodendrocyte precursor cells from various sources. In summary, we have presented a profiling methodology that can intelligently evaluate long-term state changes of stem cells by identifying subcellular/nuclear signatures at early time-points.

Conventional Assays Take Weeks to Identify Lineage Commitment



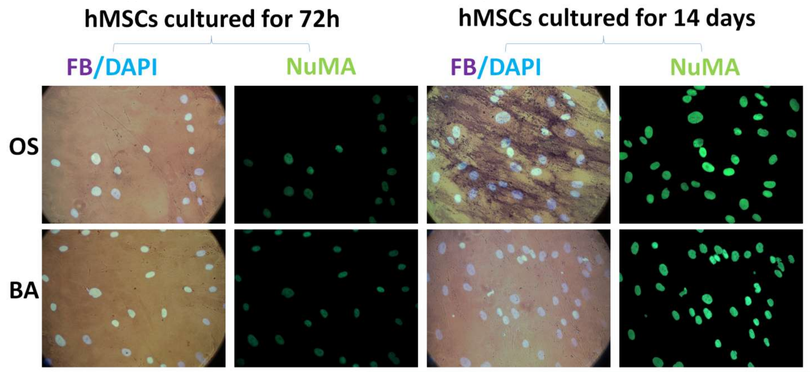
High Content Profiling Methodology

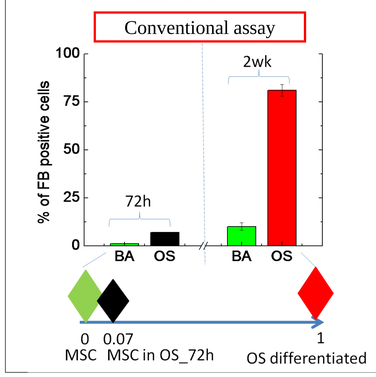
➢ **Nuclear Mitotic Apparatus (NuMA) are nuclear matrix proteins responsible for:**
1) Mitotic spindle formation
2) Nuclear structural changes during cell cycle progression
3) Nuclear cytoskeleton component, responsible for nuclear integrity
4) Chromatin organizer

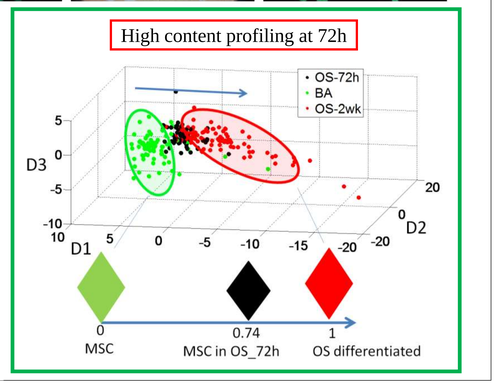


Predicting MSC Lineage Commitment

➢Human MSCs cultured in osteogenic (OS) and growth (basal, BA) medium, stained with osteogenic differentiation marker: Fast Blue (FB)

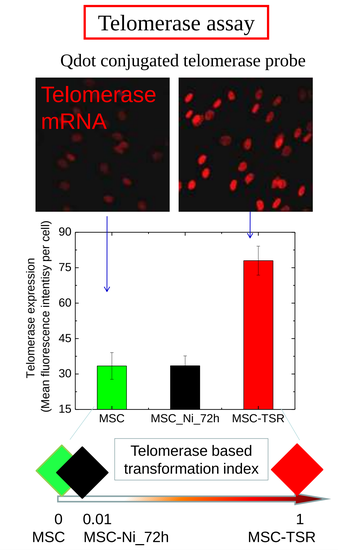


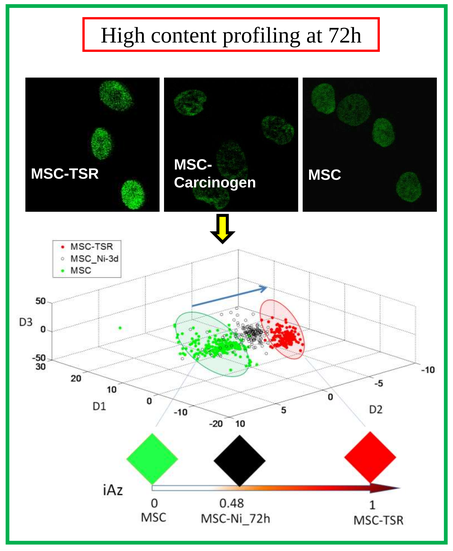




Identifying MSC Transformation

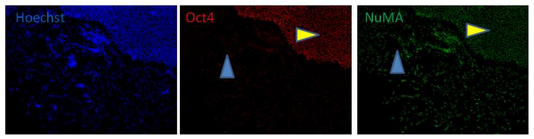
➢Carcinogenic Transformation Models
- Human MSCs transformed with oncogenes (SV40 TAg, H-Ras, hTERT)
- Human MSCs under periodic carcinogen (Nickel Sulfate) induction

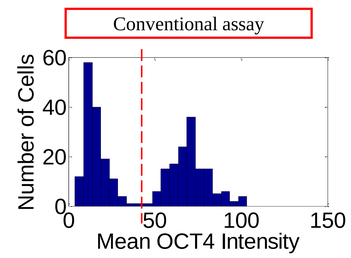


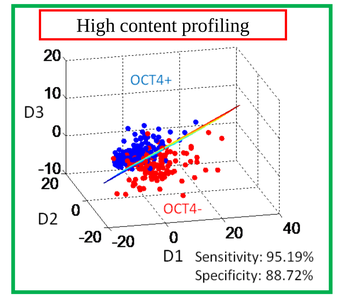


Identifying ES Lineage Commitment

➢ Human ES cells (H9) under 72h neurogenic induction

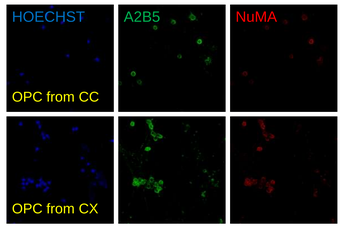


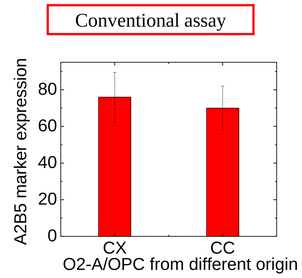


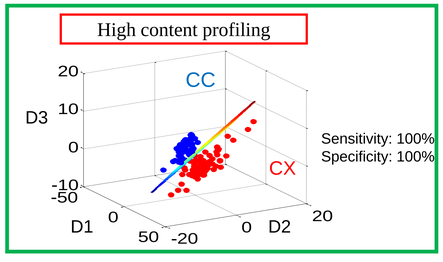


Distinguishing O-2A/OPCs from Different Sources

➢ Oligodendrocyte precursors (O-2A/OPCs) isolated from cerebral cortex (CX) and corpus collosum (CC)







Conclusions

➢A high content imaging based profiling methodology was developed to identify stem cell differentiation and transformation.

➢Utility of the methodology was demonstrated in adult stem cells, embryonic stem cells and precursor cells from different origins.

Future studies

➢ Investigate the underlying mechanism of stem cell differentiation and transformation, especially the role of cell nuclear descriptors in the differentiation/transformation process.

➢ Expand cell types, such as cancer stem cells, to demonstrate the utility of this nuclear descriptor based methodology for stem cell phenotype identifications.

Acknowledgements

Integrated Technologies for Polymeric Biomaterials, *A National Research Resource*

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