ELEMENTAL IMAGING OF HUMAN EMBRYONIC STEM CELLS

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INTRODUCTION

A critical aspect of understanding human embryonic stem cell (hESC) differentiation is regulation of protein activity. After all, it is by selectively favoring and inhibiting various proteins that cells are able to proliferate and differentiate. One way cellular systems achieve this is by changing the availability of the metal ions that activate or inhibit existing proteins. An estimated one third of all proteins bind metal ions which play numerous cellular roles including as cofactors for enzymatic reactions, structural nuclei for protein folding motifs, and various signaling pathways.(1) Little is known about the role of metals in hESCs, however zinc has been shown to be involved in the differentiation of non-pluripotent cell lines. (2)

We are elucidating the role of metals in the dynamic cellular process of hESC differentiation at a systems level and developing analytical tools applicable to any cellular system in which metal ions play such roles. Combining existing molecular and cell biology approaches with cutting-edge x-ray fluorescent capabilities at the APS, we are currently investigating dynamics in metal ion distribution across stem cell colonies upon induction of differentiation, seeking a paradigm for the role of metal ions in developmental biology. We find that cellular zinc levels may serve as a biomarker of differentiation, indirectly correlating with the expression of pluripotency markers.

EXPERIMENTAL

Human embryonic (H9) stem cells were cultured on a mouse embryonic fibroblast (MEF) feeder layer following Wicell Research Institute protocols. For XRF imaging, silicon nitride windows were coated with Matrigel before deposition of MEFs. They were fixed and prepared for imaging as we have described for other cells (4). A subset of these windows were additionally permeabilized and immunolabeled for Oct4 and stained with Hoescht dye. Using the scanning x-ray microprobe at beamline 2-ID-E at the Advanced Photon Source in Argonne, IL, cells were raster-scanned in steps of 1.0 micron collecting fluorescence spectra at each pixel. These spectra were then fitted against thin film standards using MAPS software, producing quantitative images of each element's distribution in the cell. Intentional differentiation of colonies was achieved by the addition of retinoic acid as in (5). Differentiated cells were stained and scanned in a similar method.

RESULTS

Our results demonstrate a direct correlation between integrated zinc content of cells and Oct4 immunolabeling across a colony. In spontaneously differentiating hESCs, differentiated cells are found at the center of colonies. As shown in Figure 1, cells closer to the edge of the colony contain more zinc (blue line) and less Oct4 (pink line) than cells at the colony's interior. Cellular pluripotency across the colony was illustrated by Oct4 labeling. In colonies induced to differentiate with retinoic acid (RA) as shown in Figure 2, the same pattern was observed: cells closer to the center of the colony contained more zinc and less Oct4 than near the edge of the colony, again correlating differentiation with zinc content. Pluripotent hESC colonies showed no change in zinc content across the colony.

Figure 1.

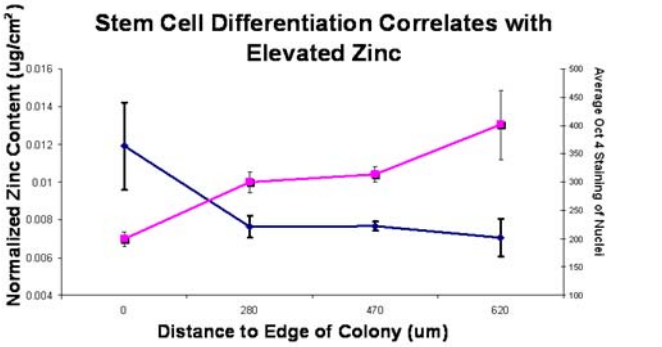
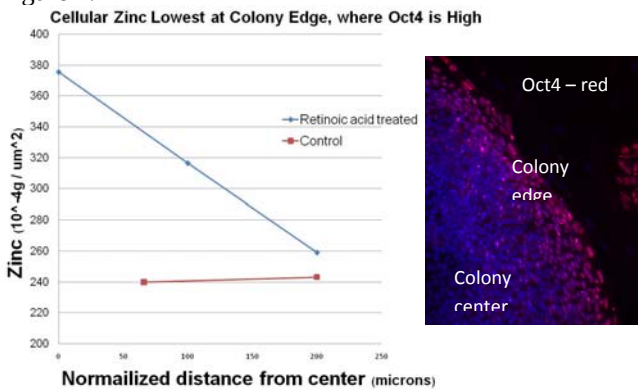


Figure 2.



These results link stem cell differentiation with increasing zinc concentration. This link may be due to cells requiring more zinc finger proteins or other zinc binding proteins for differentiation. Such a discovery would establish zinc as a control switch for maintaining pluripotency or differentiation. Ongoing work will focus on establishing a functional relationship between zinc and stem cell pluripotency.

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