

**Hyaluronan is required for generation of hematopoietic cells during differentiation of human embryonic stem cells.**

**Journal:** J Stem Cells

**Publication Year:** 2010

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**PubMed link:** 20861924

**Funding Grants:** TSRI Center for hESC Research, The Stem Cell Matrix: a map of the molecular pathways that define pluripotent cells, Ensuring the safety of cell therapy: a quality control pipeline for cell purification and validation, Collaborative Laboratory for Human Embryonic Stem Cell Research at Sanford-Burnham Medical Research Institute

**Public Summary:**

We studied the effects of Hyaluronan (HA) in differentiation of human pluripotent stem cells. Our findings indicate that endogenously produced HA contributes to the network that regulates the differentiation of hESC and the generation of mesodermal lineage in general and hematopoietic cells specifically.

**Scientific Abstract:**

Hyaluronan (HA) is an important component of the microenvironment in bone marrow, but its role in regulation of the development of hematopoietic cells is not well understood. To address the role of HA in regulation of human embryonic stem cell (hESC) differentiation into the hematopoietic lineage, we screened for genes encoding components of the HA pathway. Using gene arrays, we found that HA synthases and HA receptors are expressed in both undifferentiated and differentiating hESCs. Enzymatic degradation of HA resulted in decreased numbers of hematopoietic progenitors and lower numbers of CD45<sup>+</sup> cells generated in HA-deprived embryoid bodies (EBs). In addition, deprivation of HA resulted in the inhibition of generation of CD31<sup>+</sup> cells, stromal fibroblast-like cells and contracting myocytes in EBs. RT-PCR and immunocytochemistry revealed that HA deprivation did not influence the dynamics of OCT4 expression, but decreased the expression of BRY, an early mesoderm marker, and BMP2, a later mesoderm marker in differentiating EBs. In addition, the endoderm markers alpha-FP and SOX17 were decreased, whereas the expression of the ectoderm markers GFAP and FGF5 was higher in HA-deprived cultures. Our findings indicate that endogenously produced HA contributes to the network that regulates the differentiation of hESC and the generation of mesodermal lineage in general and hematopoietic cells specifically.

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