**The RNA binding protein HuR is critical for effective differentiation of bone marrow derived stem cells.**

Purpose

The use of stem cells to produce functional orthopaedic replacement tissue has been heavily studied. Adult stem cells, isolated as adherent monolayers from bone marrow aspirates have been seen as a particularly promising source for these applications. Whilst their ability to differentiate into a number of lineages is well characterised, the molecular mechanisms that control these processes are less well defined. One area that is particularly poorly understood in, this regard, is post-transcriptional gene control. In this study, we have examined how regulating the levels of the RNA binding protein HuR, which can control both mRNA splicing and mRNA decay, affects the differentiation efficiency of bone marrow derived stem cells.

Methods

Stem cells were isolated and expanded as adherent cells from commercially obtained human bone marrow mononuclear isolates or from murine bone marrow. Cells were expanded to passage 3-4 and then treated with small interfering RNA (siRNA) targeting HuR. Following gene knockdown, the cells were differentiated along osteogenic or adipogenic pathways in monolayer culture. Differentiation of the cells was assessed by staining of cell layers using alizarin red or oil red-O to identify calcified deposits or fat droplets respectively. Total RNA was also isolated from the cells to examine how the expression of marker genes was affected.

Results

There was evidence of reduced osteogenic differentiation in both human and murine cells where HuR levels had been knocked down by siRNA. This was accompanied by reduced expression of the osteogenic markers Runx2 and Osterix. When adipogenic differentiation was examined, no differences were observed between the control and HuR knockdown conditions.

Conclusions

Our observation of inhibited osteogenic differentiation in bone marrow derived stem cells following knockdown of HuR indicates that critical modulators of this process are controlled as a result of splice variation and/or altered rates of turnover. HuR generally acts to stabilise its target mRNAs in the cytoplasm and so knockdown would be expected to lead to increased turnover of a subset of mRNAs within the stem cells. Interestingly, knockdown of HuR does not block the cells differentiation capacity per se, as adipogenesis could still proceed. Future transcriptomic analysis will provide important insights into stem cell osteogenic differentiation by identifying genes targeted by HuR in our system.