CHIEF SCIENTIST OFFICE

CODE: TCS/18/25

RESEARCH PROJECT BRIEFING

Nanoparticle-Mediated microRNA Delivery for Increased Osteogenesis in a 3D Osteoporotic Bone Marrow Model

AIMS

In our project, we set out to determine whether we could encourage human mesenchymal stem cells (hMSCs) to differentiate into bone using specific microRNAs (miRs). This research could potentially open a new door towards treatments for bone loss, such as osteoporosis, or bone trauma/non-union fractures.

KEY FINDINGS

- We selected three different miRs to study; miR133, miR-140 and miR-211. Each one was evidenced in the literature for being involved in the regulation of MSC differentiation into bone and/or fat.
- We investigated the action of these miRs in both (i) commercially available hMSCs and (ii) our in-house isolated primary hMSCs.
- Results demonstrated no difference in gene bone markers, but a slight increase in bone protein markers at 28 days for all miRs, which was more apparent in the primary samples.
- We also compared our miR data with RNA silencing (siRNA); knockdown of the adipocyte (fat) pathway in hMSCs did not evidence with increased bone markers.

WHAT DID THE STUDY INVOLVE?

We used two different sources of hMSCS; commercial and in-house primary cells. In our primary isolation, MSCs were isolated from femoral head bone marrow, retrieved from patients following a hip replacement. Here, we attempted to isolate from healthy patients and those presenting with osteoporosis (via neck of femur fractures).

We then identified key microRNAs involved in MSC differentiation into bone, and attached them to gold nanoparticles to delivery them securely to the MSCs. Following incubation for up to 28 days, we determined key gene and protein markers for bone regeneration.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

The literature reveals a multitude of miRs that regulate the MSC fate which either induce or inhibit osteogenesis (and inhibit or induce adipogenesis). During our project:

- We selected three particular miRs; miR133, miR-140 and miR-211 involved in this process (figure 1).
- MiRs are unstable, therefore we used our established gold nanoparticle delivery platform to deliver each miR (or its antagomir – a natural blocker) to MSCs in culture.
- MSCs, both primary (healthy and ostoporotic) and commercial MSCs (purchased from Promocell) were expanded in standard DMEM media as a control, alongside osteogenic and adipogenic media.
- The miRs/antagomiRs were incubated with all samples and cultured up to 28 days.
- Gene and protein markers for MSC 'stemmness', fat and bone were assessed at days 7 and 28 (figure 2 & 3).

We discovered that we could enhance bone protein markers at day 28 using all of our miR treatments. This was more pronounced in our primary healthy and ostogenic MSC samples.

We did not see any striking difference between normal and osteogenic patient's MSCs in terms of miR treatments, but subtle differences in their potential to differentiate were noted.

In the final stages of our project, we compared delivery of naked miR treatments (i.e. no gold nanoparticles) with delivery of siRNA to knock down two key adipogenic pathways, with the aim of promoting osteogenesis. Whilst gene knockdown worked well, we did not see any marked difference in bone formation.

Figure 1. A schematic illustrating the varied points at which microRNAs (miRs) can influence MSC differentiation into bone. Note the actions of miR133, miR-140 and miR-211, which were utilised in this project (adapted from Hu, L., et al. Int. J. Mol. Sci., 19, 2018).

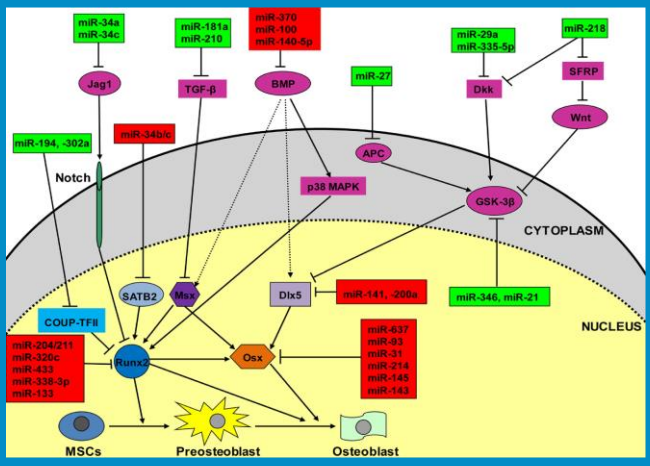


Figure 2. MSCs (both primary and commercial) were cultured in normal (untreated) adipogenic and osteogenic media. All samples were then incubated with miR treatments for up to 20 days. Gene and protein markers for MSC 'stemmness', fat and bone were assessed. Here, In Cell Western was used to assess protein markers.

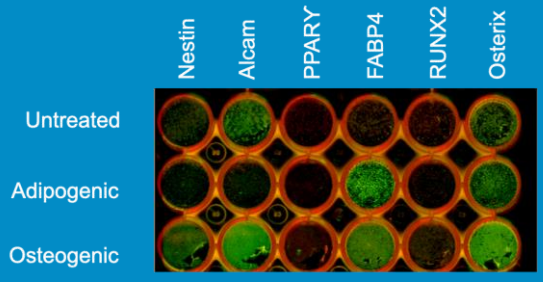
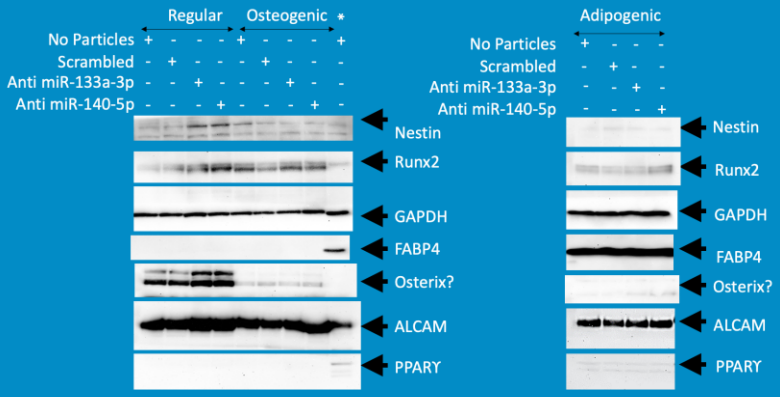


Figure 3. Western blots were carried out for all samples (explained in figure 2) to verify In Cell Western results. Here, primary MSCs cultured from osteoporotic patients were assessed for MSC, fat and bone markers at day 28.



WHAT IMPACT COULD THE FINDINGS HAVE?

- Our project has shown that there are subtle differences in the potential for osteoporotic patient bone marrow to differentiate into bone. This may in turn affect the potency of treatments aimed at enhancing bone formation.
- The gold nanoparticles platform did not perform as well as in our pilot work (published evidence of miR-31 inhibition and subsequent increases in bone protein markers). Therefore we adapted and moved to study the potential for naked miR and siRNA delivery. We aim to publish this work in the coming year due to its academic impact.

HOW WILL THE OUTCOMES BE DISSEMINATED?

We have presented parts of the data from this project at several national conferences including the Tissue and Cell engineering Society (TCES) and British Orthopaedic Research Society (BORS).

The difference between the delivery of three different naked miRs and two siRNA approaches will be published in a scientific journal during 2022/23.

We also aim to include parts of this work in our outreach and engagement in local secondary schools.

CONCLUSION

We have identified that miRs can be used with varying effect to enhance bone formation in primary healthy and osteoporotic MSCs. It was further noted that the combined effect of using multiple miRs was not as effective at using single miR treatments, potentially due to off target effects. The treatments need to be further optimised before moving forward with potential animal studies.

RESEARCH TEAM & CONTACT

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