Enhanced chondrogenic potential of miR-221 and Slug depleted human MSCs

P229

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INTRODUCTION

Human Mesenchymal Stromal Cells (hMSCs)-based tissue engineering is generally regarded as one of the most promising approaches for cartilage regeneration. Nevertheless, suitable protocols for the *in vitro* manipulation of stem cells for the production of implantable constructs are yet to be found. In this context, our work is focused on the development of innovative strategies to induce efficient chondrogenic differentiation of hMSCs for their clinical use in cartilage repair. **Recently, miR-221 and Slug transcription factor have emerged as negative chondroregulatory molecules(1,2) and their downregulation may therefore be required for chondrogenic differentiation.** Nevertheless, the possibility to induce hMSCs chondrogenesis by manipulating the expression of these regulators has not been explored before.

AIM

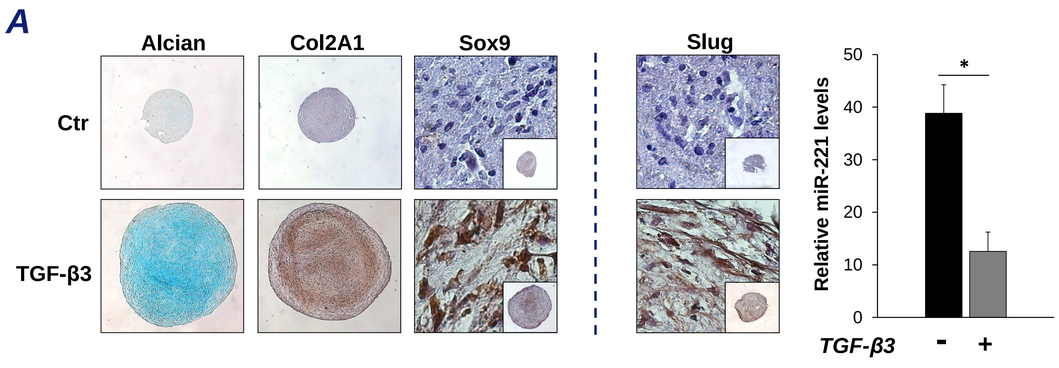
We investigated if the inhibition of miR-221 and Slug transcription factor could be sufficient to address hMSCs from Wharton's Jelly (WJMSCs) towards a chondrocyte-like phenotype, in the absence of traditional differentiating agents, such as TGF-β

HIGHLIGHTS

miR-221 or Slug depletion has a pro-chondrogenic effect in hMSCs and triggers the early steps of chondrogenesis, in the absence of TGF-β

METHODS & RESULTS

1) Expression of Slug and miR-221 during chondrogenesis

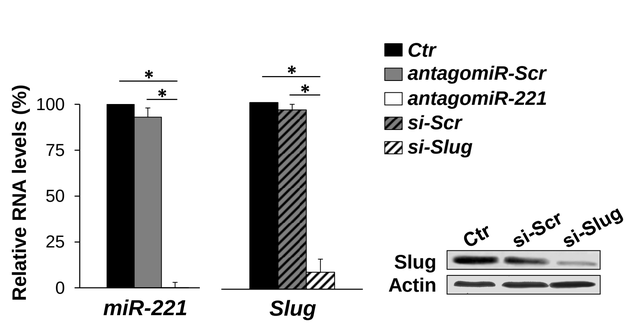


A: *Evaluation of Slug and miR-221 expression after TGF-β3 induced chondrogenesis of WJMSCs, by immunohistochemistry and qRT-PCR, respectively.* ***B:*** *miR-221 and Slug expression in two WJMSCs groups with opposite outcome of chondrogenesis after TGF-β3 exposure (*p<0.05).*

Chondrogenesis of WJMSCs induced by TGF-β3 determines inhibition of miR-221, while Slug protein is unexpectedly upregulated (A). miR-221 downregulation is required for chondrogenesis, while **Slug upregulation is a mere effect of TGF-β treatment and may counteract the differentiation process** (B).

May chondrogenesis be induced in the abscence of TGF-β?

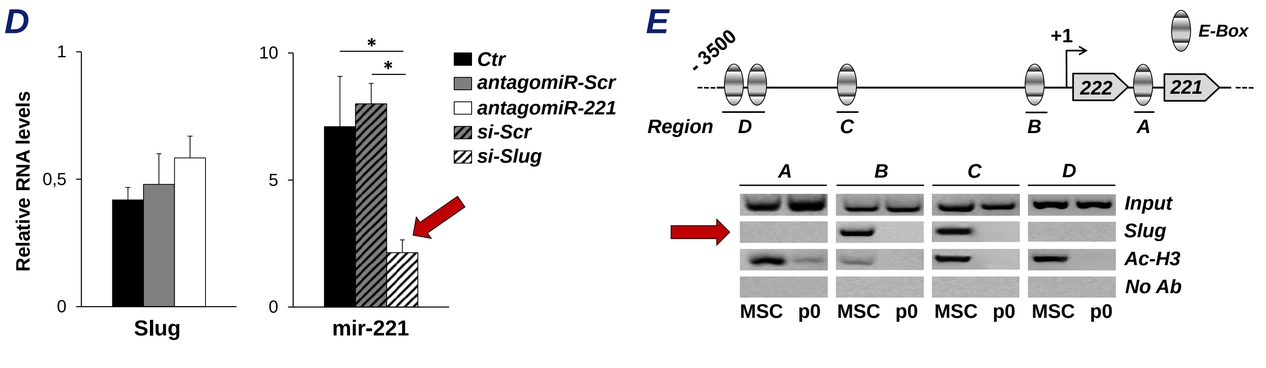
2) Experimental strategy: gene silencing



C: *miR-221 and Slug expression was inhibited in WJMSCs, using specific antagomiR and siRNA molecules. Monolayered cells were transfected twice a week since the day after plating and the efficiency of gene silencing was assessed at day 6 by qRT-PCR and Western Blot analysis (*p<0.05).*

The expression of miR-221 and Slug was inhibited in WJMSCs by a gene silencing approach. At day 6, a strong efficiency of knockdown was achieved (C).

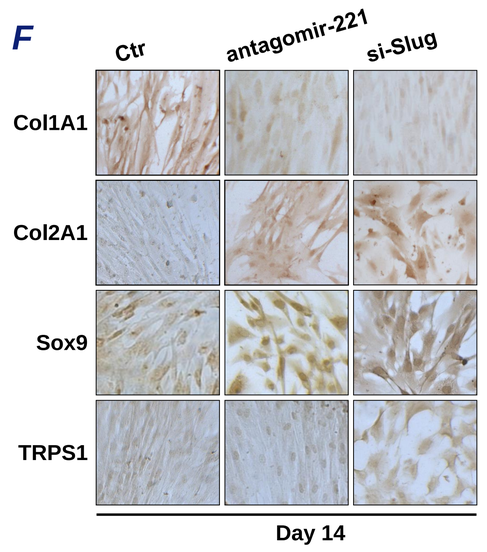
3) A novel Slug/miR-221 circuit regulates chondrogenesis



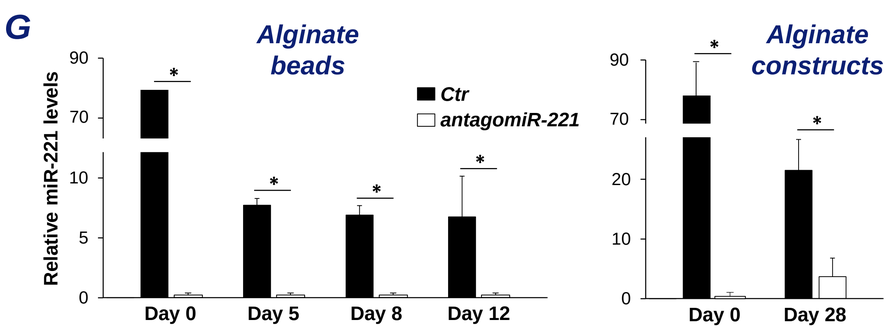
Slug expression is not influenced by miR-221 knockdown, but Slug inhibition determines a decline in the levels of miR-221 (D). Slug is recruited «in vivo» at the miR-221 promoter in WJMSCs (E) → **Slug directly stimulates the expression of miR-221 in hMSCs.**

Exposure to antagomiR-221 or si-Slug reduced the levels of Col1A1, caused the appearance of Col2A1, and strongly increased Sox9 (F). si-Slug only also caused an increase of TRPS1. **Slug or miR-221 depletion drives hMSCs towards chondrogenesis in the absence of TGF-β.**

D: *qRT-PCR analysis of Slug and miR-221 expression after miR-221 and Slug silencing, respectively (*p<0.05).* ***E:*** *Analysis of the "in vivo" recruitment of Slug at the miR-221 promoter by chromatin immunoprecipitation in WJMSCs and mature p0 chondrocytes.* ***F:*** *Effect of miR-221 and Slug knockdown on protein expression in WJMSCs, investigated by immunocytochemistry.*



4) *Work in progress*: combination with alginate-based scaffolds

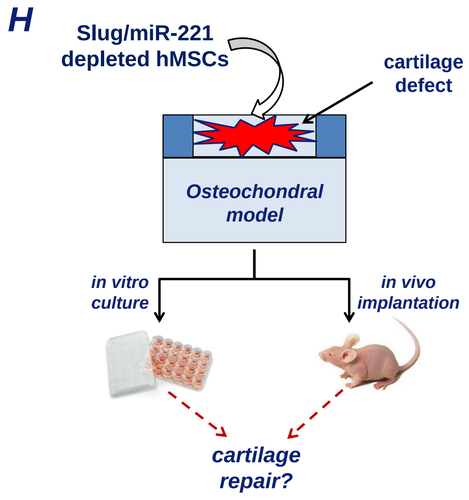


G: *Assessment of miR-221 expression by qRT-PCR after embedding of miR-221 depleted human MSCs from bone marrow (BMSCs) in alginate beads or alginate constructs, up to 28 days (*p<0.05).*

The efficiency of miR-221 knockdown is maintained after embedding of miR-221 depleted BMSCs in alginate beads or constructs, up to 28 days post-encapsulation.

CONCLUSIONS & PERSPECTIVES

- miR-221 or Slug silencing in hMSCs induces differentiation towards the chondrogenic lineage, in the absence of chondrogenic inducers, such as TGF-β. Our engineered hMSCs represent an **innovative stem cell-based product to be tested *in vivo* for its cartilage regenerative potential(3).**
- The combination of the engineered hMSCs with a biocompatible scaffold like alginate preserves the efficiency of gene silencing, demonstrating the **feasibility of this approach for the generation of tissue engineered constructs.**
- On-going work is aimed at **evaluating the ability of alginate embedded–engineered hMSCs to trigger reparative processes in the context of a cartilage defect** (H), *in vitro* and *in vivo*, by using a recently developed osteochondral model(4).



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