INTRODUCTION
Spinal cord injury is usually irreversible, largely because the central nervous system has very limited regenerative potential. Therefore, based on an extensive self-renewal capacity that stem cells have, various stem-cell based therapies have been tested in basic research, preclinical and clinical studies. Due to their developmental potential and ability to proliferate indefinitely, induced pluripotent stem cells (iPSCs) stand as a prospective tool for the generation of patient-specific cell derivatives used for regenerative therapies. Yet, significant challenges remain in their clinical development, including epigenetic abnormalities associated with induced pluripotency. It is crucial to elucidate the regulatory pathways that determine cell fate, and this research will elucidate some of the mechanisms by which a chromatin-remodeling factor, Suv39h1, modulates iPSC fate decisions.

MOTIVATION
A loss-of-function RNA interference screen for 22 chromatin-remodeling enzymes conducted to study the influence of chromatin modifiers on iPSC production demonstrated that enzymes modifying histone 3 lysine 9 (H3K9) have varying impacts on the reprogramming process. These findings evoke the presence of complex mechanistic events underlying the reprogramming process, especially as they affect the involvement of H3K9 methyltransferases, including Suv39h1. Suv39h1 knockdown (KD) specifically substantially boosted the rate and efficiency of reprogramming, but functional consequences for epigenetic stability remain unidentified. This study seeks to elucidate whether silencing of Suv39h1 promotes epigenetic modifications to the aim of cell therapy. Understanding the molecular mechanisms by which Suv39h1 impairment facilitates induction of iPSC cells will be beneficial to optimizing reprogramming conditions.

METHODS

-Determine if the mechanism of increased efficiency of iPSC generation in Suv39h1 KD cells is due to the overall loss of the protein or specifically loss of the methyltransferase activity

Whether addition of chaetocin, a small-molecule inhibitor of Suv39h1 catalytic activity, at doses ranging from 1 μM to 10 μM, can increase reprogramming efficiency of human fibroblast cells will be tested. Reprogramming procedures and measurements of efficiency will be performed as described by Onder et al., 2012 in all experiments. To further confirm that the methyltransferase activity of Suv39h1 is responsible for the increased reprogramming efficiency caused by Suv39h1 knockdown, either a wild type Suv39h1 protein or a catalytically mutant Suv39h1 protein following shRNA treatment will be added back and whether reprogramming efficiency decreases upon addition of the wild type or catalytic mutant protein tested. To allow for protein expression of wild type Suv39h1 or the Suv39h1 catalytic mutant and evade knockdown of the transfected gene by shRNA, a viral vector expressing wild type or catalytic mutant mouse Suv39h1, which will be resistant to shRNA against human Suv39h1, will be transfected into a human fibroblast cell line. Reduced reprogramming efficiency will be assessed post-transfection after selecting colonies based on human embryonic stem cell morphology. These colonies will be stained for Tra-1-60, a marker of reprogrammed human stem cells, to monitor complete reprogramming of human somatic cells. The percentage of Tra-1-60 positive colonies will then be quantified to determine reprogramming efficiency.

-Distinguish possible gene expression changes upon Suv39h1 KD and elucidate the global chromatin changes promoted by Suv39h1 impairment

Genome-wide expression profiling using DNA microarrays following Suv39h1 shRNA KD in mouse fibroblasts will be performed. These gene expression profiles will be compared to mouse fibroblasts without Suv39h1 impairment and be used for genes that are significantly upregulated in Suv39h1 KD iPSCs. Chromatin immunoprecipitation followed by sequencing (Chip-seq) for H3K9 methylation in iPSCs derived from fibroblasts with and without Suv39h1 impairment will also be used. Large decreases in H3K9me3 at the promoters of genes known to be involved in pluripotency and those that are highly expressed in ES cells will be looked for using a 2.5 fold change in expression level as a threshold.

EXPECTED RESULTS

Fig 1. Suv39h1 inhibition enhances reprogramming efficiency

A. Fold change in the reprogramming efficiency of human fibroblast cells treated with chaetocin at the indicated concentrations for 21 days. Data correspond to the mean +/- s.d.; n = 5. *P < 0.001 compared to untreated fibroblasts.

B. Fold change in the reprogramming efficiency of human fibroblast cells infected with 2 independent Suv39h1 shRNAs or co-infected with shRNA-1 and a vector expressing an shRNA-resistant wild-type or catalytically dead mutant Suv39h1. Data correspond to the average and s.e.m.; n = independent experiments. *P < 0.01 control shRNA-expressing empty vector

DISCUSSION
If chaetocin administration improves the efficiency of iPSC cell generation, the conclusion that inhibition of catalytic activity of Suv39h1 is responsible for the increased reprogramming efficiency observed following Suv39h1 KD will be supported. This conclusion will be confirmed by adding Suv39h1 shRNA and chaetocin to mouse fibroblasts and assessing reprogramming efficiency, in which case KD of Suv39h1 in the presence of chaetocin should not improve iPSC production further, suggesting that inactivation of the methyltransferase activity, as opposed to loss of Suv39h1 protein, is indeed the epigenetic factor responsible for increased reprogramming efficiency. If impairment of the enzymatic activity of Suv39h1 does not eliminate its associated repressive activities, the hypothesis will need to be adjusted to accommodate the likelihood that there are genes that Suv39h1 is able to modulate that are independent from its methyltransferase function. In particular, if this is the case, Suv39h1’s repressive activities may indicate a role for a scaffolding function of the protein at target genes, as has been proposed for the Salvador (sav) gene in orchestrating cell-cycle exit and apoptosis. If genes whose expression was affected by depletion of Suv39h1 are identified, these data will support the existence of a group of genes regulated by Suv39h1. Identification of a set of targets that are affected by Suv39h1 will be candidates for causing the increased reprogramming efficiency caused by loss of Suv39h1. If the data support the existence of promoters having reduced levels of H3K9me3, this would suggest the presence of a more permissive chromatin structure that, perhaps by promoting transcriptional activation of the core reprogramming factors, may favor optimization of the reprogramming condition.

SUMMARY POINTS
iPSCs represent a promising strategy for regenerative medicine, but the epigenetic instability induced after the forced expression of reprogramming factors remains a serious concern when using them for cell therapy. This research tests the hypothesis that silencing of Suv39h1 will facilitate iPSC induction by promoting epigenetic modifications. Comparative global gene analysis of fibroblasts that have been treated with a small molecular inhibitor of Suv39h1 will be used to identify genes with increased levels of H3K9me3 as assessed by Chip-seq. Genes identified in this approach will be silenced by RNAi to confirm their involvement in iPSC differentiation. These studies offer preliminary insights into the mechanism by which Suv39h1 contributes to cell fate decisions in iPSCs- a dimension of the use of the reprogramming methods for clinical application that has not been fully explained.

SELECTED REFERENCES