Characterising the HMGN proteins expression, and their role, during neuronal differentiation of mouse P19-ECCs in a defined adherent culture system

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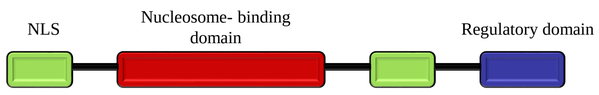
Introduction

HMGN family members are nucleosome-binding proteins that modulate chromatin structure and regulate DNA-dependent function such as replication, repair, and transcription. HMGN1 and HMGN2 have been shown to play important roles in early development. *Hmgn1* knockout mouse embryos have defects in corneal maturation, increased tumourigenicity and impaired ability to repair damaged DNA. However, *Hmgn2* homozygous knockout seems to be embryonic lethal in mice. Further studies revealed that the expression level of HMGN proteins is tightly related to the differentiation process of both *Xenopus* and mice. Moreover, the expression of HMGN1 and HMGN2 must decrease during erythropoiesis, chondrogenesis, and myogenesis in order to ensure proper differentiation. Here, we demonstrate that the expression of HMGN1 and HMGN2 proteins also decreased in the terminal stage of neuronal differentiation *in vitro*, using an adherent culture system in embryonic carcicinoma cells (ECCs, P19). Thus, this adherent culture system provides a model to investigate the role of HMGN proteins and epigenetic processes during neuronal differentiation.

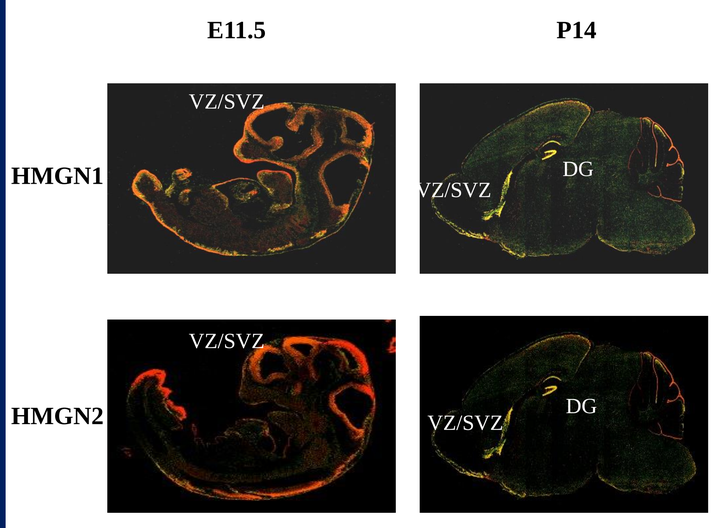
We also studied the effects of *Hmgn* knockdown on the neuronal differentiation of ECCs *in vitro*. The expression levels of various neural stem cell and neuronal genes were altered following *Hmgn* knockdown, suggesting that HMGN proteins are important for accurate neuronal differentiation and function.

HMGN proteins



HMGN proteins regulate transcription by binding to nucleosomes and altering chromatin structure. They can alter the pattern of histone modifications, compete with linker histones, and interact with transcription factors.

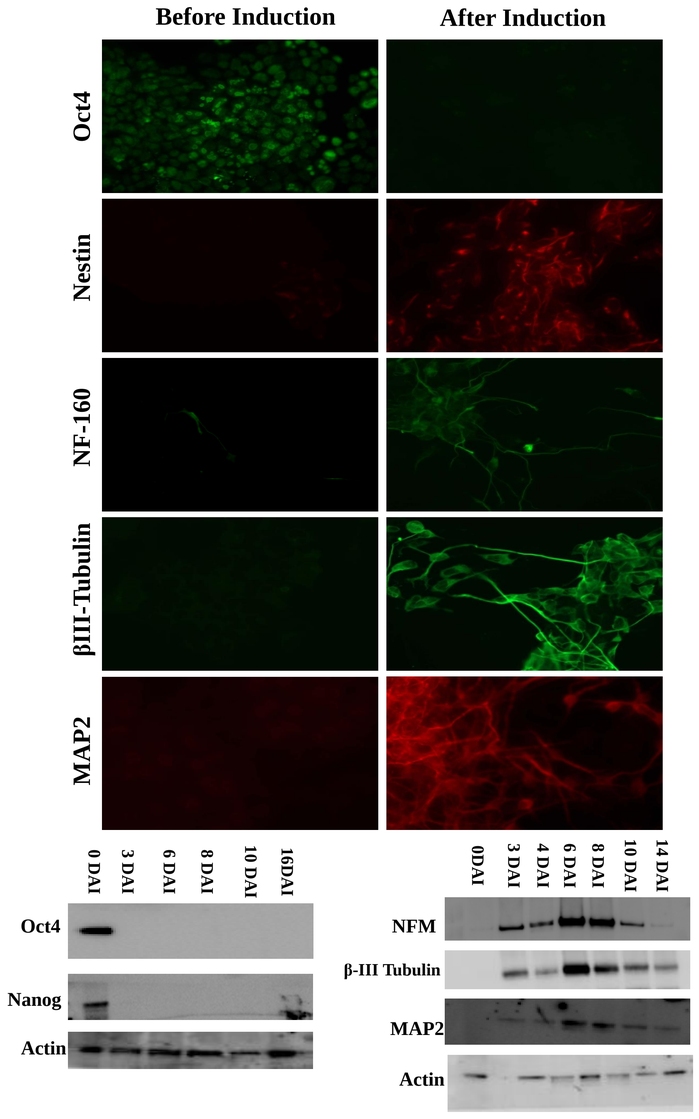
HMGN1 and HMGN2 expression in the mouse brain



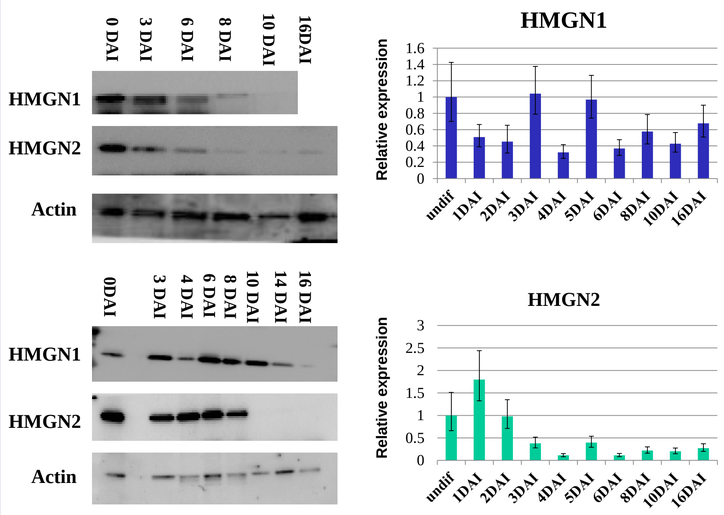
VZ: ventrivcular zone; SVZ: subventricular zone; DG: dentate gyrus (in the hippocampus).

• Data from the Allen Brain Atlas indicates that HMGN1 and HMGN2 mRNAs are highly expressed in active neurogenic regions of the developing and adult mouse brain, including the subventricular zone and the dentate gyrus of the hippocampus.

Adherent monolayer culture efficiently drive NPCs and neurons production from P19 ECCs

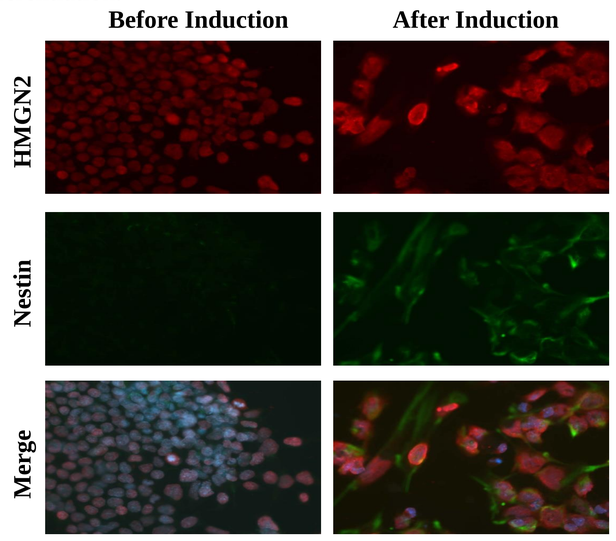


HMGN1 and HMGN2 seem to be reduced during neuronal differentiation *in vitro*



Preliminary data from two different experiments suggest a reduction in the expression of HMGN1 and HMGN2 observed at the protein level by Western Blot (left) after the neural induction. Both proteins are found in high levels in the undifferentiated ECCs, and are almost lost in terminal stages of differentiation.

Consistently with the Western Blot results, the relative expression of HMGN1 and HMGN2 mRNAs show a potential reduction during the process. The error bars correspond to three technical replicates from the same sample.

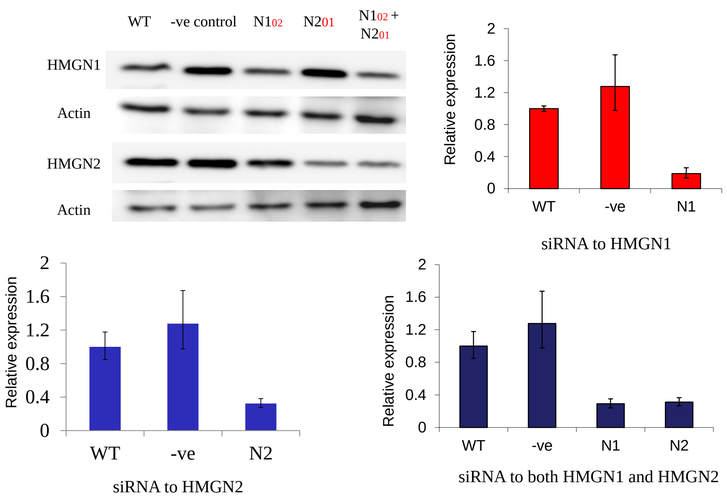


The majority of Nestin-positive cells highly express HMGN levels.

The potential role of HMGN1 and 2 in the neural differentiation process

•In order to asses the role of the HMGN proteins during the neural differentiation process, a siRNA kockdown system was employed.

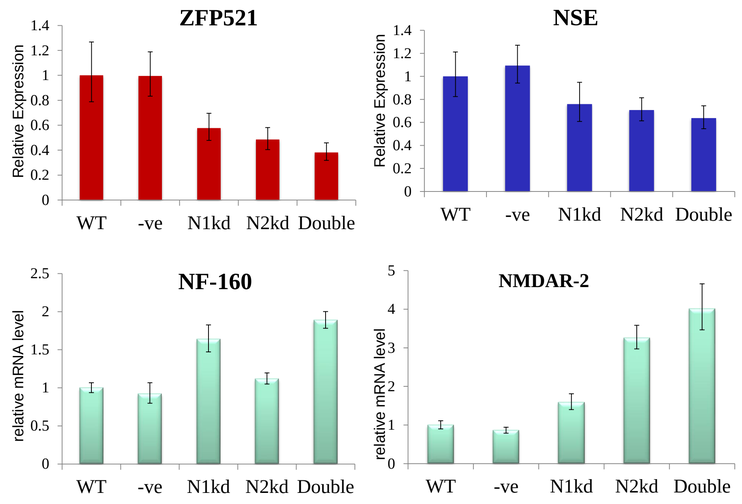
• The effect of the reduction in the levels of HMGN proteins was evaluated using an embryoid body-based neural induction system after 3 days of plating.



HMGN1 and HMGN2 RNA levels were knocked down by more than 75% in cells transfected with $N1_{02}$ and $N2_{01}$ siRNAs respectively. In the double knockdown experiments, both HMGN1 and HMGN2 RNA levels were knocked down by more than 65% compared to wild type cells. Both protein levels show the same trend.

The reduction in the levels of HMGN1 and/or HMGN2 affects the expression of certain early neural induction and neuron-specific genes at day 3 after plating:

• NSE, ZFP521 expressions are down-regulated.
• NF-160 and NMDAR-2 expressions are up-regulated.



The results represent the average of the relative expression of each gene tested per triplicate from one biological replicate.

Conclusions and future work

• The most abundant members of HMGN protein family are highly expressed in neural stem cells where they potentially play an important role in this tissue-specific stem cell biology and in their differentiation process.

• Interestingly, in the late stages of the differentiation process these proteins were found in less abundance. This is in accordance with previous studies showing that the expression of HMGN1 and HMGN2 decrease during erythropoiesis, chondrogenesis, and myogenesis in order to ensure proper differentiation. Over-expression of HMGN proteins can be performed during neural induction to address the idea.

• Further work is required to determine whether knocking down/out HMGNs during neural differentiation induce changes in gene expression that can affect the process.

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