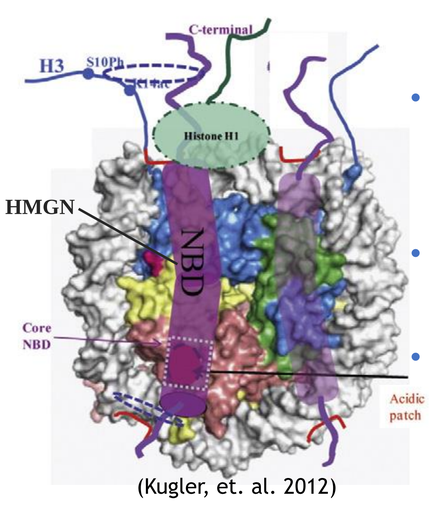
Nucleosome-binding HMGN proteins inhibit stem cell differentiation down the neuronal lineage

S Garza-Manero, A Sindi, M Bailo, G Mohan, O Rehbini, V Jeantet, AG West, KL West

Institute of Cancer Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK

Introduction



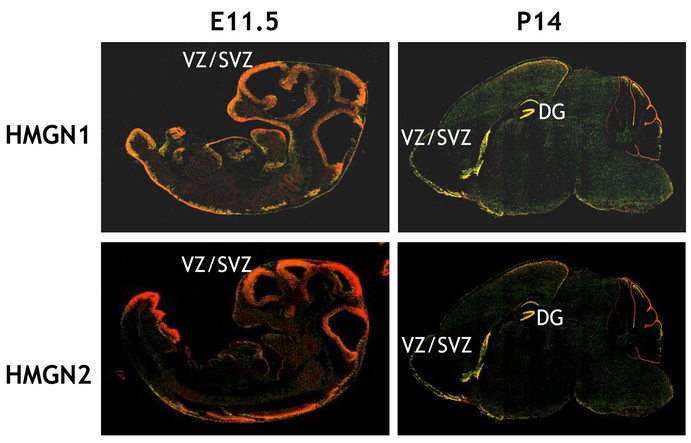
(Kugler, et. al. 2012)

HMGN proteins

- The high mobility group nucleosome binding (HMGN) proteins bind specifically to nucleosomes through their nucleosome binding domain (NBD, 1,2).
- The C-terminal region of the proteins interacts with the histone tails influencing histone modifications (3).
- The HMGNs compete with linker histone to modulate chromatin architecture (4,5) and therefore DNA-dependent functions such as transcription.

Developmental regulation of the HMGNs

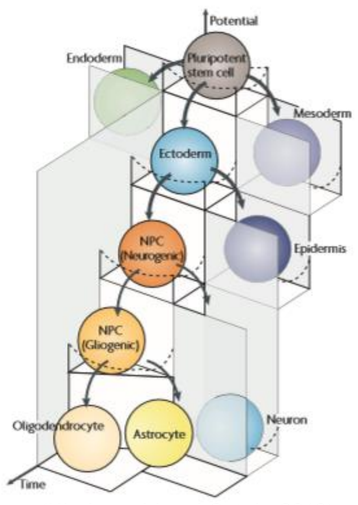
- The two major members of the family, HMGN1 and HMGN2, are abundant in embryonic tissues and progressively downregulated as differentiation proceeds (6).
- However, tissue-specific stem cells retain high levels of these proteins (7).
- The *Hmgn1* and *Hmgn2* mRNAs are enriched in well characterised neural stem cell niches of the developing and adult brain.



(Allen Brain Atlas Database)

Neural development

- Studies *in vitro* suggest the neural identity as the default pathway for an embryonic stem cell (9, 10).
- Once stablished the neuroectoderm, cortical development present three sequential phases; expansion, neurogenic and gliogenic (11).
- The neural stem cells lose neurogenic potential along development, whereas acquiring the capacity to generate glia cells (11).
- Fate determination of neural stem cells, ie whether they become neurons or glia, is influenced by extrinsic instructive factors and intrinsic transcriptional programs; thus epigenetic players potentially modulate their interaction (12, 13).



(Hirabayashi and Gotoh, 2010)

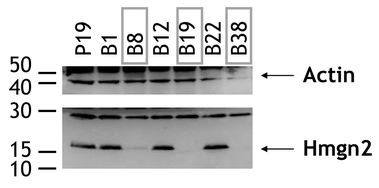
General Aim

Investigate the role of the HMGN proteins in embryonic stem cell differentiation down the neural lineage.

Results

Stable embryo carcinoma cell lines lacking the Hmgn2 protein

- To get insights about the HMGN function in the embryonic stem cell biology and differentiation down the neural lineage, the P19 mouse embryo carcinoma cell line was used as parental line.
- The CRISPR/Cas produces targeted single or double strand breaks in the genomic DNA by recruiting the Cas9 nuclease to the region of choice through a guide RNA (gRNA) complementary in sequence (14, 15).
- A modified version of the Cas9 with nickase activity (16) was used to introduce mutagenesis in the ATG start codon of the *hmgn2* gene.
- Three different Hmgn2-knockout monoclonal lines were derived.



The Hmgn2 protein inhibits stem cell differentiation down the neuronal lineage

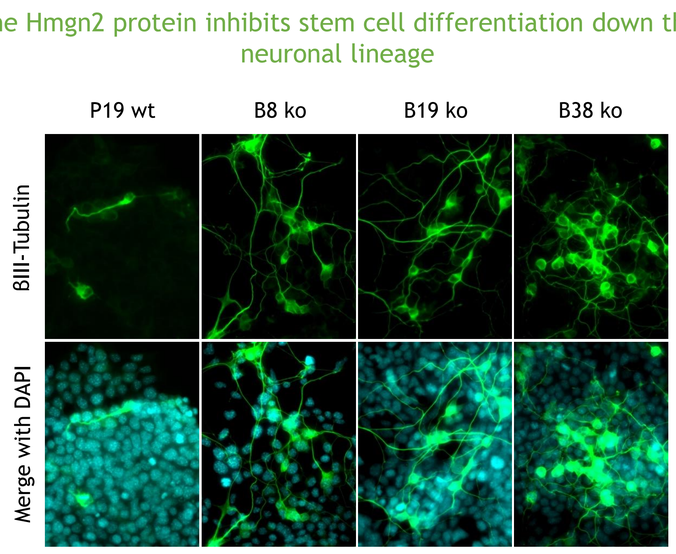


Figure 1. Presence of differentiated cells in the Hmgn2-knockout cultures. The P19 wt and Hmgn2-ko cells were immuno-stained for the neuronal marker βIII-Tubulin. The three Hmgn2-ko cultures show higher numbers of neurons compared with the P19 wt culture.

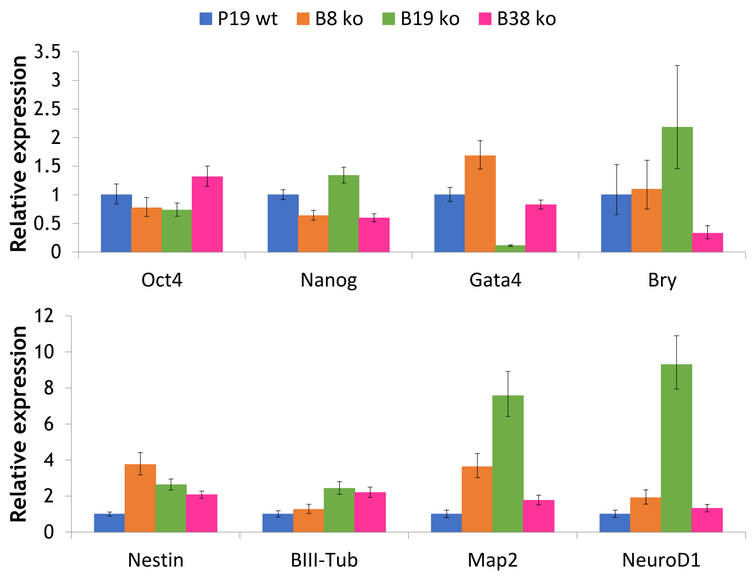


Figure 2. Differentiation of Hmgn2-knockout cells is exclusively down the neuronal lineage. qRT-PCR analysis show higher levels of expression of pro-neuronal genes in the Hmgn2-ko cells such as Ngn1, Mash1 and NeuroD1. There is a trend of reduction in the expression of the pluripotency genes Oct4 and Nanog. The lack of change in the endoderm and mesoderm markers, Gata4 and Brachyury, indicate that the spontaneous differentiation is specific down the neuronal lineage.

The Hmgn2-knockout cells retain higher neurogenic potential

Adherent culture onto laminin-coated dishes and serum free media (17)

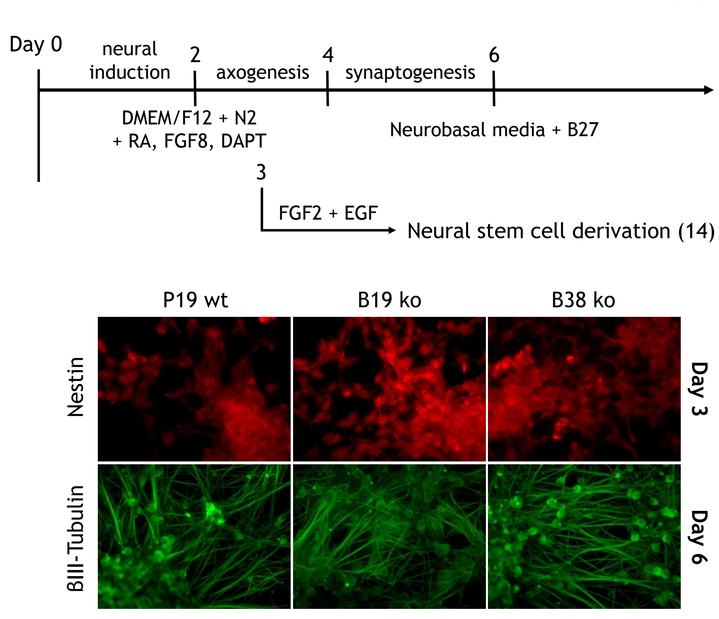


Figure 3. Neuronal induction of Hmgn2-knockout cells. The P19 wt and Hmgn2-ko cells were induced to differentiate into the neuronal pathway as described (17). No evident changes were observed between them and was possible to derivate neural stem cell lines from all of them. The neural stem cell marker Nestin revealed high production of these cells at day 3 and the βIII-Tubulin labelling identify neurons forming complex networks by day 6.

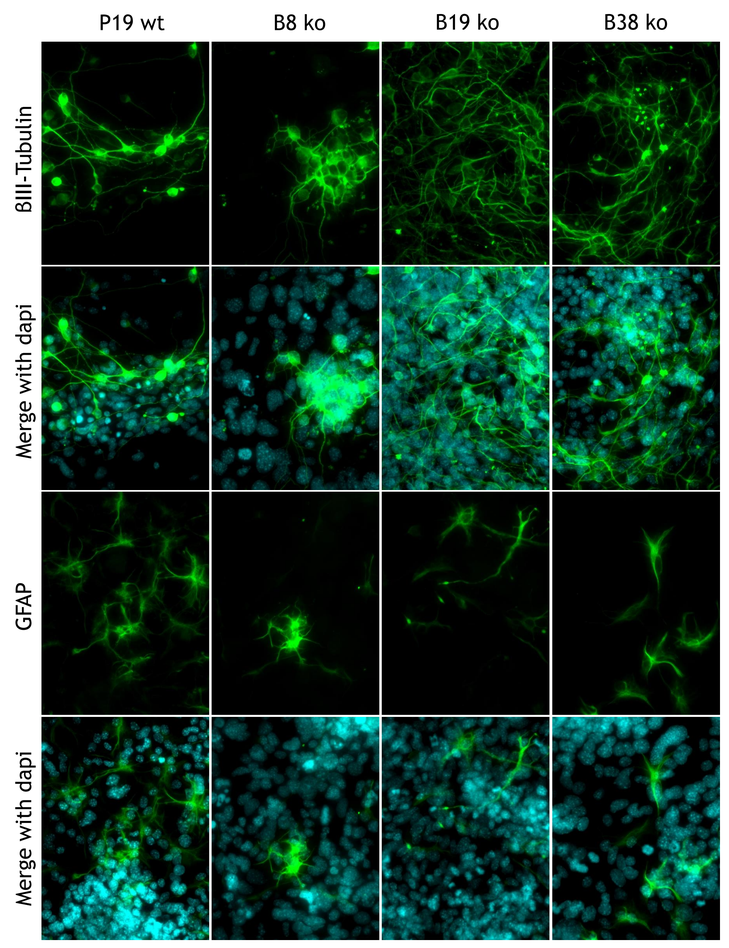


Figure 4. Hmgn2-knockout derived neural stem cells retain higher neurogenic potential.
Neural stem cells were derived from P19 wt and Hmgn2-ko lines. In all cases, the neural stem cells demonstrate neurogenic and gliogenic potential. However, as a preliminary observation, the Hmgn2-ko cells produce fewer glial cells than the P19 wt neural stem cells which is in agreement with previous reports (19).

Conclusions

- The Hmgn2 protein inhibits stem cell differentiation down the neuronal lineage.
- The lack of the Hmgn2 protein leads to a spontaneous differentiation of stem cells into neurons and no other cell types.
- The neural stem cells derived from the Hmgn2-ko lines retain higher neurogenic potential than the P19 WT derived.
- The HMGN proteins are required for proper decision-making during embryonic and neural stem cell differentiation.

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