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Prion protein in ESC regulation

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A large number of studies have analyzed the putative functions of the prion protein (PrP^C) in mammals. Although its sequence conservation over a wide range of different animals may indicate that this protein could have a key role in prion diseases, an absolutely accepted involvement has not been found so far. We have recently reported that PrP^C regulates *Nanog* mRNA expression, the first non-redundant function of PrP^C in embryonic stem cells (ESC), which translates into control of pluripotency and early differentiation. Contrary to what is believed, the other two members of the prion protein family, Doppel and Shadoo, cannot replace the absence of PrP^C, causing the appearance of a new embryoid body (EB) population in our in vitro culture. The similarities between EB and an early post-implantation embryo suggest that this might also occur in vivo, enhancing the importance of this finding. On the other hand, our data may support the hypothesis of a relationship between the loss of PrP^C function and neuronal degeneration in prion diseases. A reduction in brain stem cells pluripotency after PrP^C is misfolded into the pathological conformation (PrP^{Sc}) could lead to a delay or a disappearance of the normal brain damage recovery.

Key words: prion protein, differentiation, pluripotency, embryoid bodies, primordial germ cells, integrins, prion diseases

Abbreviations: EB, embryoid body; PrP^C, prion protein; PGC, primordial germ cell; NPCs, neural precursor cells; LIF, leukemia inhibitory factor; KO, knock out; ESC, embryonic stem cells; WT, wild type

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Although an important function for the cellular prion protein (PrP^C) has been proposed, it still remains unclear. PrP^C plays a major role not only in the central nervous system, but also in other tissues. In addition to the brain, it has been detected in several non-neuronal tissues, including lymphoid cells, lung, kidney, heart, gastrointestinal tract, muscle, mammary glands, etc.[1,2] Recently, there is new evidence that it may also have possible implications in tissue morphogenesis,[3,4] tissue and stem cells regeneration[5] and embryogenesis.[6] However, our understanding of its physiology remains poorly detailed. Regarding the embryo development, it is known to be a highly intricate matrix of genes that require a precise timepoint coordination to achieve the adequate biochemical signaling. Until now, several groups of genes have been considered to play an essential role in this process, but those that are involved in pluripotency and differentiation are of particular interest. The maintenance or the loss of stemness is one of the main activities the cell has to carefully manage, bearing in mind that the embryo is a differentiating system.

Current knowledge places *Sox2*, *Oct3/4*, *Nanog* and *Stat3* among the most important genes involved in the maintenance of stem cell pluripotency.[7-9] PrP^C has also been related to this property since it is expressed at almost 1.5 times higher in embryonic stem cells (ESC) than in somatic cells,[7] reaching a maximum of 6.2 times in the first two weeks of differentiation.[8] The discovery of its essentiality in supporting self-renewal in haematopoietic stem cells[9] confirms this point. An involvement in long term proliferation of cancer cells (glioblastoma, breast, prostate and gastric cancer) has been described as well,[10,11] similar to that exhibited by ESC, thus there was a need to study how PrP^C is able to regulate this process.

In our work, we demonstrated that PrP^C influences the expression of genes involved in ESC self-renewal. Particulary, we found

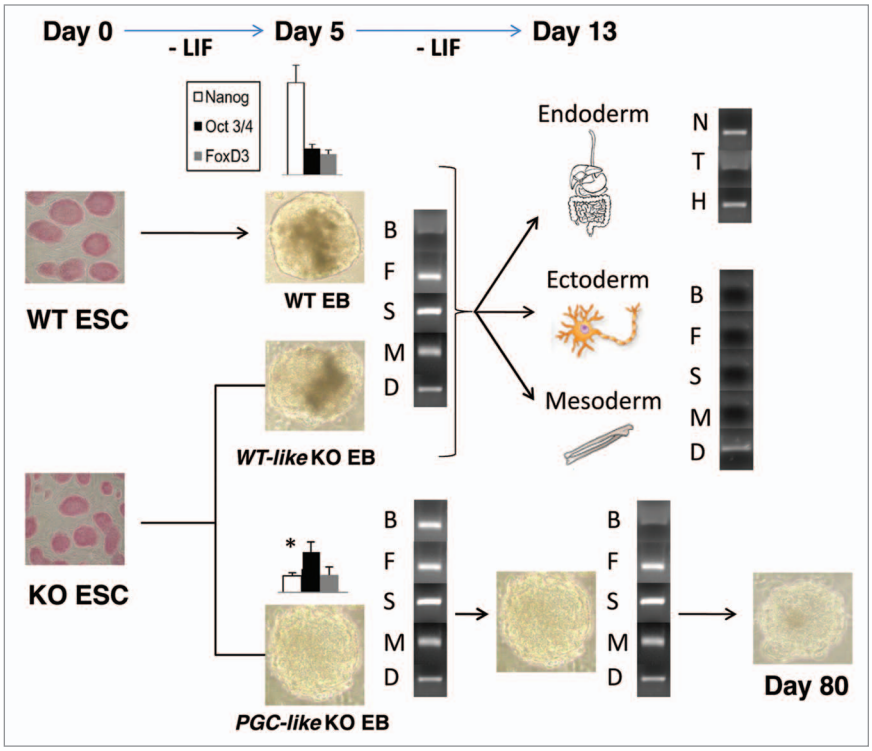

Figure 1. PrP^C function during early ESC differentiation. Scheme showing gene expression and morphological differences during differentiation of WT and PrP^C-null (KO) ESC. In the KO line, two EB populations (*WT-like* KO and *PGC*-like KO EBs) appeared when Leukemia Inhibitory Factor (LIF) was removed from the medium. The *WT-like* KO EBs developed in a similar way to WT EBs, showing a different *Nanog* expression on Day 5 of differentiation than the *PGC*-like KO EBs. This led to a complete differentiation into the three embrionary layers by Day 13, since all the early ectoderm [*Nestin* (N)], mesoderm [*Brachiury* (T)] and endoderm [*Hnf3* (H)] markers were present and the majority of the PGC makers analysed [*Bmp4* (B), *Fragilis* (F), *Stella* (S), *Mvh4* (M) and *Dazl* (D)] were absent. On the other side, the *PGC-like* KO EBs expressed all the PGC makers analysed during the entire early differentiation, and this population retained its morphology even longer (80 days).

that PrP^C transcription regulates the mRNA expression of the important pluripotent marker gene *Nanog* in early ESC differentiation[12] (**Fig. 1**). Furthermore, the absence of PrP^C also provoked the appearance of a subpopulation of embryoid bodies (EBs) in the PrP^C-null (KO) culture at that time, which retained the expression of primordial germ cell (PGC) markers for a longer period (**Fig. 1**) and maintained a high pluripotency level.[12]

Even though *Nanog* is not included between the minimal combinations of genes required to obtain iPS from fibroblasts,[13,14] it is a crucial protein. Several studies report how the overexpression of this gene releases ESC pluripotency from the dependence of *Stat3* stimulation, also reducing and retarding the ESC differentiation.[15,16] On the contrary, the ablation leads to a failure in the specification of the early embryo pluripotent cells and to a parietal and visceral ESC segregation.[15,16] *Nanog* is also important because it is a member of the feedback cluster that exists along with *Oct3/4* and *FoxD3*,[17,18] both described to play a key role in pluripotency.[19,20] These data enhance the value of the relationship between *Prnp* and *Nanog* in animal development, moreover considering that it seems to take place in foetal and neonates gonads.[12]

On the other hand, we have described that this association was partially mediated by integrin-β5.[12] Integrins are heterodimeric transmembrane proteins that are involved in cell adhesion and signal transmission mechanisms, forming part of processes such as cell death, migration, differentiation and proliferation.[21,22] This result was supported by some studies, which described that integrins, e.g., integrin-β1, were related to *Nanog* expression[21] and several others that showed how PrP^C was also involved in integrins regulation, e.g., in the development of embryonic cell adhesion via E-cadherin.[23] Thus, our findings suggest a complex interaction matrix in which integrins play a significant role. Further experiments should clarify if this role may be linked to the facilitation of protein interaction or to signal modulation as a feedback control.

In our in vitro experiments, we included the derivation of ESC into EBs because of their documented similarities with an early post-implantation embryo.[24] Unexpectedly, a new EB population appeared from Day 5 in the *KO* line culture, persisting even longer (80 days) [12] (**Fig. 1**). It might suggest the possibility of preexistence populations of ESCs in the theoretical pure initial culture, since a variety of ESC based on low or high *Nanog* levels has been previously described in reference 17. The *Prnp* knockout could have promoted the differentiation of one of those specific KO ESCs when LIF was removed from the medium, being revealed on Day 5 as a special EB subpopulation which expressed PGC markers (*PGC-like* KO EB) [12] (**Fig. 1**).

In a prion context, this is the first non-redundant function described for the PrP^C, since the other members of the prion family (Doppel and Shadoo) are not able to compensate the effects derived from PrP^C disappearance.[12] Although many studies have tried to elucidate the physiological role of the protein, their conclusions were contradictory or not conclusive.[25] Furthermore, KO animals, in which Doppel is not artifactually upregulated, did not show any significant alterations.[26,27] This unfinished exploration is impeding the resolution of an important question, since PrP^C changes its conformation to acquire pathological properties (PrP^{Sc}),[28] leading perhaps to a loss of PrP^C functionality. The consequence could be a role in the pathogenesis of prion diseases, for instance, reducing post-injury regeneration. Stella et al. show a delay in muscle reparation due to the absence of PrP^C, maybe via tumor necrosis factor (TNF) α, a molecule described to mediate either neuronal cell death or neuroprotection,[29] not only in neurons[4] but also in muscle.[3] Furthermore, it was demonstrated that the differentiation of neural precursor cells

(NPCs) was delayed when the PrP^C was not functional, although it did not affect their final morphology or the final tissue morphology.[5] In this scenario, additional defects in differentiation, late activation or prolonged proliferation in the "repairing" cells could critically worsen the pathological circumstances, preventing an improvement. Thus the discovery of a reduced *Nanog* expression in the KO EBs[12] might imply a critical decrease in the NPCs quantity and pluripotency in vivo in a prion infected brain. Accordingly, it could lead to a lower response against the lesions caused by the common harmful agents, e.g., reactive oxygen species, and to a possible explanation for the progressive tissue degeneration in these unique pathologies.

In conclusion, PrP^C has a key function during early embryogenesis and adult regeneration, with this last feature suggesting an explanation for prion pathogenesis. The response against external damage could be diminished as a consequence of the PrP^C conformational change that occurs in prion diseases. Hence, it remarked the relevance of the PrP^C function studies, which allow us to increase our comprehension of the prion disease behaviour. Importantly, our work also proposes the timing when the putative PrP^C role might take place. All KO systems are perfectly viable probably because of a compensation phenomenon, thus these studies have to be carried out at the moment when these compensatory pathways are still not established, in our particular case, during early differentiation.

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