PDE2A for Mouse Liver Development

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cAMP and cGMP are intracellular signaling molecules produced in response to a plethora of extracellular signals in order to coordinate cellular metabolism, proliferation, differentiation and apoptosis. Phosphodiesterases (PDEs) are the enzymes that hydrolyze cAMP and cGMP in order to end or to limit the responses to these signals.

To date 11 PDE families (named PDE1 to PDE11) have been identified across each cell type expressed in a peculiar pattern. They enclose 21 genes that codify approximately 100 enzymes that form a redundant network ensuring the compensation of activity in case of alteration of activity or lack of expression of one of the members. PDE2A, a cAMP-hydrolyzing enzyme, represents the exception to this picture, as PDE2A knockout is embryonic lethal. Knockout embryos show that the lack of the enzyme has the greatest impact on the development of the heart and of the liver, which is no longer able to assume its hematopoietic role. The increase of the intracellular cAMP level and the downregulation of the anti-apoptotic gene Bcl2 might explain the loss of integrity in the PDE2A knockout liver niche that compromises the hematopoietic function and maturation.

Keywords: Phosphodiesterase 2A ; cAMP ; apoptosis ; hematopoiesis ; fetal liver

1. Introduction

During most of prenatal development, the fetal liver (FL) provides the specific niche required for hematopoiesis. Its components, hepatoblasts, resident macrophages, fibroblasts, vascular smooth muscle, stromal and endothelial cells participate with extracellular matrix (ECM) and humoral factors to its generation [1]. In the mouse, at embryonic day 11.5 (E11.5), hematopoietic stem cells (HSC), after their formation in the yolk sac, aorta-gonad-mesonephros region, and placenta, migrate in FL attracted by chemical signals. In FL between E12 and E16, HSC dramatically increase in number and differentiate into mature hematopoietic cells, which are necessary to sustain the ongoing growth of the embryo [2]. Around E16, the hematopoietic function of FL starts to decline in favor of the bone marrow and the liver begins to acquire specific metabolic functions, which include carbohydrate and lipid metabolism [3].

A plethora of genetic alterations affecting liver development has been reported to compromise hematopoiesis. By examining in vitro proliferation and differentiation of the hematopoietic committed progenitors it is possible to distinguish if defects are being housed in the niche or in the hematopoietic precursors themselves [4][5][6]. Phosphodiesterases (PDEs) are essential components of cellular signaling that regulate the response to several stimuli (hormones, neurotransmitters) by modulating intracellular levels of cyclic nucleotides [7]. PDEs are expressed in different combinations in tissues and in cellular compartments allowing the proper regulation of cyclic nucleotides signaling [8]. Some PDEs are cAMP or cGMP-specific or, as in the case of phosphodiesterase 2A (PDE2A), they can hydrolyze both cAMP and cGMP. In the adult mouse, PDE2A has a widespread tissue distribution but brain, heart, liver and adrenal gland show the greatest level of expression. Mouse development is compromised in *PDE2A* knockout embryos [9]. PDE2A deficiency causes cardiac defects, associated to the increase of cAMP level and of ICER, a cAMP-dependent transcriptional repressor, which in turn reduces the expression of genes essential for cardiomyocyte differentiation [10]. The role of PDE2A in liver development and hematopoiesis has been not yet established, but in the adult the role of cyclic nucleotides in regulating protein kinase activity and gene transcription for key liver functions is well documented [11][12].

Here we report a new role of PDE2A in FL. The absence of this enzyme severely affects the development of this organ at the structural, cellular and molecular levels with severe consequences on its role in prenatal hematopoiesis.

2. Results

PDE2A knockout embryos die at E15.5 with evident cardiac and hepatic defects (FIGURE 1). At the time of death, their liver weighs only 25% of the wild type counterpart. At a microscopical level such livers display loss of the normal structure: the cells are less densely packed, the central vein in the lobule is absent and the erythroblasts, easily detected at E14.5 in

normal livers are scarcely represented. The manifestation of such phenotype is probably due to the increase in intracellular cAMP concentration that in *PDE2A*$^{-/-}$ livers is significantly higher compared to the levels detected in the liver of *PDE2A*$^{+/+}$ embryos. The high cAMP level can deregulate the cellular pathways it controls. As a matter of fact, overall, the results suggest that in vivo PDE2A contributes to hepatoblast, stromal and endothelial cells differentiation/survival.

In particular, in *PDE2A*$^{-/-}$ embryos:

-Liver hypoplasia is caused by apoptotic increase rather than decrease of cellular proliferation. Hence, the PDE2A/cAMP signaling may play a role in protecting liver cells from apoptotic factors during development.

-The absence of PDE2A induces apoptosis of different cell types that contribute to the liver niche and this in turn affects the differentiation of hematopoietic stem cells that have colonized fetal liver of *PDE2A*$^{-/-}$.

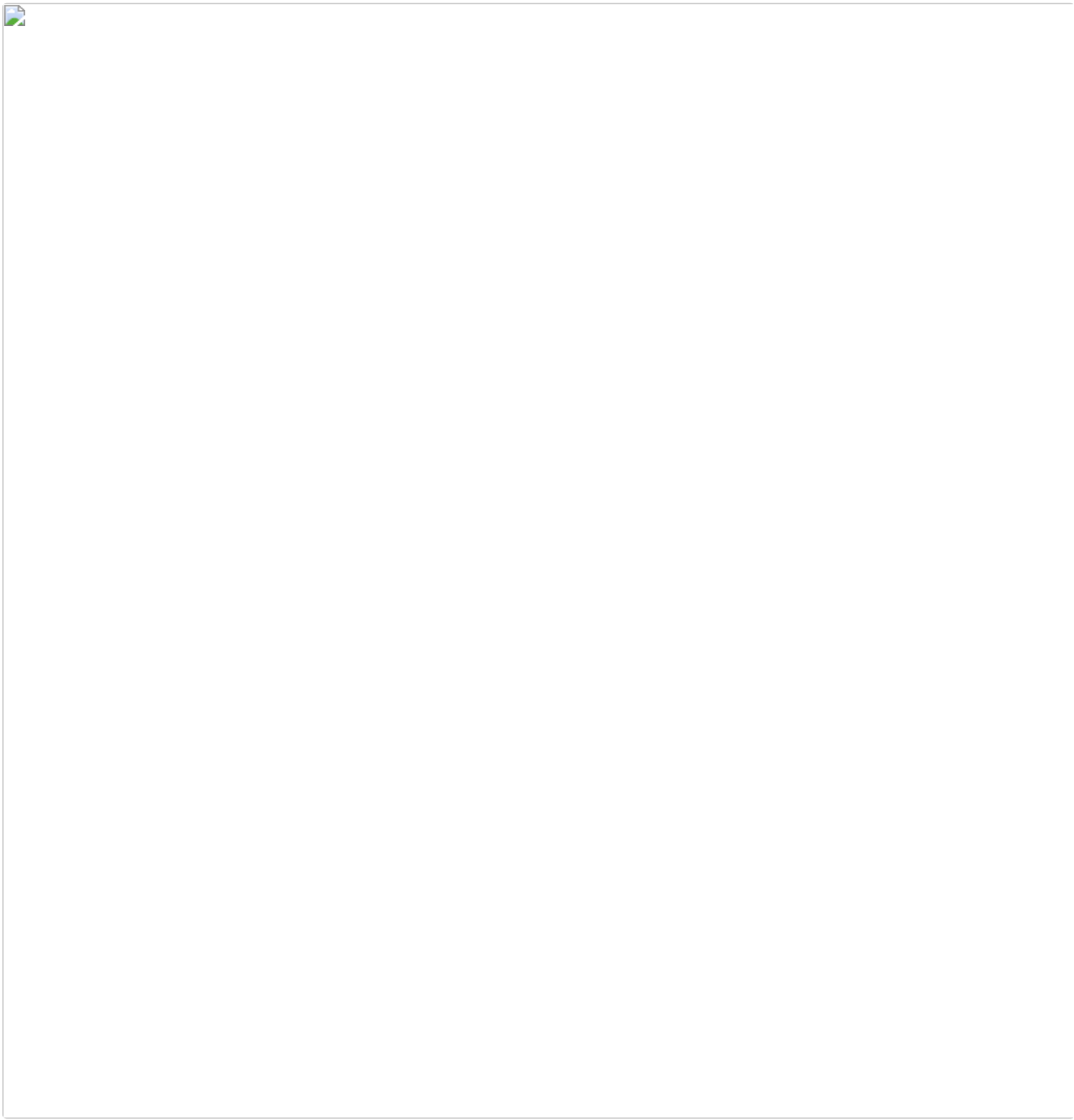

Figure 1. Morphological defects in liver of PDE2A$^{-/-}$ embryos. Volume rendering visualization of coronal sections obtained from E14.5 PDE2A$^{+/+}$ and PDE2A$^{-/-}$ embryos and scheme of potential mechanism causing liver and hematopoietic defects in PDE2A$^{-/-}$ embryos. Sections were chosen referring to the stomach lumen (LS) volume. H heart, L liver, PC peritoneal cavity, IS interlobular space, MV mesencephalic vesicle, SG submandibular gland, RM roof of midbrain, NP nasopharynx.

3. Conclusion

In this study, the results show that the lack of PDE2A causes profound defects in early liver development. At the time of death, livers are hypocellular because of apoptosis and embryos are pale because the differentiation of mature blood cells from their progenitors is defective.

PDE2A deficient embryos share similarities with other mice models in which gene disruption causes abnormal liver development. One example is offered by *XBP-1* knockout mice [6]. XBP1 is a major component of the unfolded protein response (UPR) of the endoplasmic reticulum. Knockout mouse embryos die at mid-gestation because the hematopoietic progenitors do not generate mature blood cells for the inadequate environment in the hypoplastic liver, but they differentiate normally in vitro. However, XBP-1 is a cAMP-dependent factor and indeed *XBP-1* mRNA is upregulated in *PDE2A* mutants implying that this factor is unlikely to be responsible for the phenotype of *PDE2A*$^{-/-}$ embryos. However, it remains possible that cAMP level in *PDE2A*$^{-/-}$ embryos affects the activity of other UPR components. *PDE2A* and *XBP-1* knockout embryos share a decreased expression of αFP, an early marker, which promotes the growth of liver cells and may contribute to the reduced liver size in both models. Furthermore, *PDE2A*$^{-/-}$ embryos have reduced expression of albumin and of several liver specific transcription factors, essential to establish liver function (such as cEBPα, cMet, HNF1α, HNF4α).

The absence of PDE2A cannot be compensated by other phosphodiesterases and this accounts for the high level of cAMP observed in the liver of mutant embryos underlining the importance of this enzyme in balancing cAMP signaling. Agents that elevate cAMP stimulate apoptosis by activating PKA in a variety of systems, including thymocytes [13], immortalized primary granulosa cells [14][15], human mammary carcinoma cells [16] and various normal and transformed T and B cells [17]. In other cases, PKA activated by cAMP blocks apoptosis. Examples include aged neutrophils [18], macrophage cell lines exposed to exogenous nitric oxide [19], ovarian follicles [20]and T cells [21]. A high level of cAMP could promote apoptosis not only by activating PKA, but also by directly deregulating the transcription of cAMP-responsive genes such as ICER that represses the transcription of several CRE (cAMP responsive regulatory element) containing genes [22]. The cAMP/ICER system is upregulated in the liver and in the heart of *PDE2A*$^{-/-}$ embryos. As in the liver, the heart displayed increased apoptosis and modulation of several genes necessary for cardiogenesis [10]. Several examples indicate that ICER can induce apoptosis by downregulating the Bcl2 anti-apoptotic gene [23][24]. In *PDE2A*$^{-/-}$ livers, ICER is upregulated and Bcl2 is downregulated, suggesting a mechanism by which liver cells undergo apoptosis.

In conclusion, in the mouse, the lack of PDE2A through the increase of cAMP could activate apoptosis by affecting directly both ICER and Bcl2 genes; however, the involvement of alternative second messengers as for example variation of intracellular calcium concentration is possible [25]. The widespread cell apoptosis causes the loss of the cellular microenvironment essential for the differentiation of hematopoietic stem cells. The consequent failure of erythrocytes production results in an anemia that is probably the principal cause of death for *PDE2A*$^{-/-}$ embryos.

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