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Review

Signaling by Retinoic Acid in Embryonic and Adult Hematopoiesis

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Abstract: Embryonic and adult hematopoiesis are both finely regulated by a number of signaling mechanisms. In the mammalian embryo, short-term and long-term hematopoietic stem cells (HSC) arise from a subset of endothelial cells which constitute the hemogenic endothelium. These HSC expand and give rise to all the lineages of blood cells in the fetal liver, first, and in the bone marrow from the end of the gestation and throughout the adult life. The retinoic acid (RA) signaling system, acting through the family of nuclear retinoic acid receptors (RARs and RXRs), is involved in multiple steps of the hematopoietic development, and also in the regulation of the differentiation of some myeloid lineages in adults. In humans, the importance of this RA-mediated control is dramatically illustrated by the pathogeny of acute promyelocytic leukemia, a disease produced by a chromosomal rearrangement fusing the RARα gene with other genes. The aberrant fusion protein is able to bind to RARα target gene promoters to actively suppress gene transcription. Lack of function of RARα leads to a failure in the differentiation of promyelocytic progenitors. In this review we have collected the available information about all the phases of the hematopoietic process in which RA signaling is involved, being essential for steps such as the emergence of HSC from the hemogenic endothelium, or modulating processes such as the adult granulopoiesis. A better knowledge of the RA-mediated signaling mechanisms

can contribute to the knowledge of the origin of many pathologies of the hematopoietic system and can provide new clinical avenues for their treatment.

Keywords: retinoic acid; hematopoiesis; hemogenic endothelium; bone marrow; acute promyelocytic leukemia

1. Introduction

Adult hematopoiesis is the complex process of generation of blood cells from multipotent progenitors. The fine regulation of the number of cells of each hematopoietic lineage and its fitting to the physiological and pathological conditions of the organism requires of sophisticated control mechanisms involving a large number of signaling systems acting in the bone marrow, the niche of the hematopoietic stem cells (HSC) [1–3]. On the other hand, embryonic hematopoiesis originates both the early cohorts of blood cells necessary for proper development and the long term HSC [4,5]. Differentiation of blood cells and HSC from the mesoderm, particularly from the hemogenic endothelium through an endothelial-hematopoietic transition (EHT), also requires fine regulation by a number of signaling mechanisms [6]. In this paper, which forms part of a special issue of *Journal of Developmental Biology* devoted to the developmental mechanisms regulated by retinoids, we will review the current knowledge about how retinoic acid (RA) is critically involved in both, embryonic and adult hematopoiesis. We will pay special attention to the developmental processes in which RA is playing a function for differentiation of long term HSC. We will also describe how a failure in the RA-mediated signaling mechanisms can originate severe pathologies of the hematopoietic system in humans.

2. Transgenic Mouse Models for RA Signaling and Hematopoiesis

RA is synthesized from its precursor molecule, retinaldehyde (derived from dietary retinol, *i.e.*, the vitamin A) by retinaldehyde dehydrogenases (RALDHs). There are three of these enzymes in mammals, RALDH1, 2 and 3. RALDH1 deficient mice are viable [7] and RALDH3 knockout display respiratory failure and die a few hours after birth [8]. RALDH2 is the most important RA-synthesizing enzyme in mesodermal tissues, and in fact RALDH2 deficient murine embryos die by the stage E10.5 [9]. These mutant embryos show abnormal vascular and hematopoietic development, as described below and in Table 1.

Retinoic acid signaling is mediated by two families of nuclear receptors, RARs and RXRs [10,11]. There are three members of each family, known as α, β and γ. Each of them can be expressed as two isoforms generated by alternative splicing. Functional nuclear retinoid receptors are frequently composed of RXR and RAR heterodimers, although RXRs can form homodimers and also heterodimers with other nuclear receptors such as the vitamin D receptor or PPAR. These receptors usually perform repressor roles when they are unbound, and transcriptional activators when bound to the ligands. Loss of function of RXRβ and RXRγ in mice have no consequences for early and mid-gestation [12], although a half of the RXRβ knockout mice die at birth, showing no apparent hematopoietic defects

(Table 1) [13]. In contrast, RXRα is lethal for the embryo between E13.5 and E16.5, originating cardiac, ocular and hepatic abnormalities [14,15], although defects in early hematopoiesis of RXRα knockout embryos have not been described. This is supported by conditional inactivation of RXRα in hematopoietic progenitors using an IFN-inducible MxCre/RXRα-floxed system. These mice show normal hematopoiesis despite lack of compensatory upregulation of RXRβ [16].

Mice deficient for RARα, β and γ, survive until birth [17–20], but both, RARα and γ knockout mice exhibit early postnatal lethality when both isoforms are disrupted [17–19]. Homozygous RARβ mutants are viable and fertile, but they show growth retardation and vertebral malformations [21].

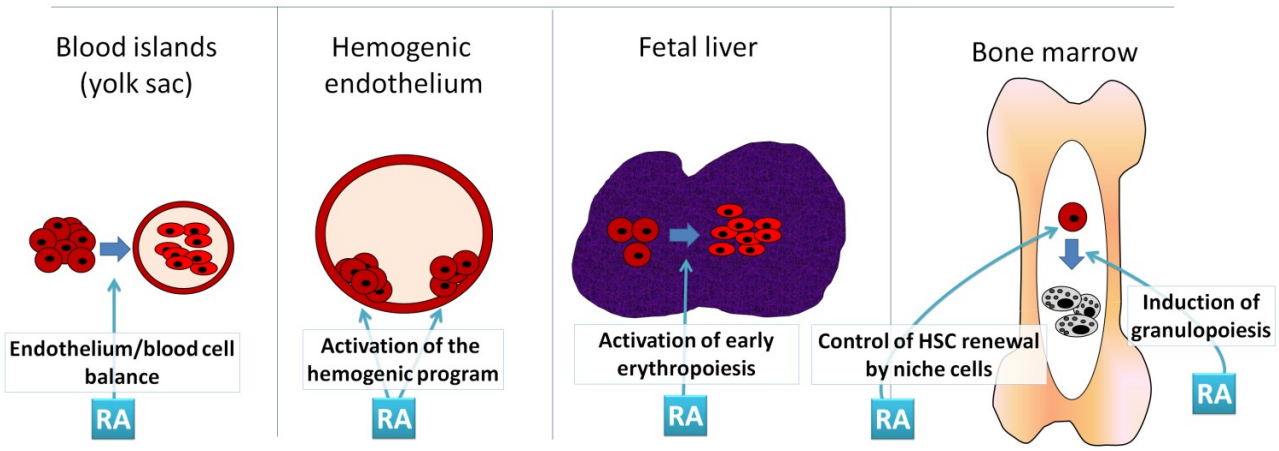
The viablity of mice deficient for RARα or RARγ, despite their reduced lifespan, demonstrates that these receptors are not essential for developmental hematopoiesis. However, as described in the section devoted to adult hematopoiesis, both receptors play important modulatory roles during the differentiation of the blood cell lineages in the bone marrow. Particularly, adult RARγ-KO mice show a defect in the number of HSC, they are anemic and develop myeloproliferative syndrome, as described below [22–24]. On the other hand, the double mutant for RARα1/RARγ die by the stage E18.5 or shortly after birth. Their bone marrow cells differentiate normally *in vivo* and form colonies *in vitro* with the same frequency than single mutants or wildtype littermates. However, double mutant colonies remain blocked at the myelocyte and, to a lesser extent, at the metamyelocyte stages, whereas erythroid and macrophage differentiation was not affected [25]. The double mutant RARα/RARγ (all isoforms) die at midgestation with multiple defects but with liver hematopoiesis apparently normal [19,20].

Taken together, the analysis of the different RAR mutant phenotypes suggests a dispensable role for RA during embryonic hematopoiesis. However, the triple mutant, where the three RAR types (α, β, γ) would be disrupted, has not been done, so we cannot exclude the possibility of an active and essential role of RA during this process [20,25–27]. Other phenotypes of mutant mice for different elements of the RA signaling pathway are described in Table 1.

3. RA and Developmental Hematopoiesis

In the mouse embryo, hematopoiesis starts around E7-7.5, when primitive blood cells and endothelial cells appear in the mesoderm of the yolk sac. These clusters of endothelial and blood cells connect with the endothelium-lined channels that are developing in the embryo. When the heart starts to beat, the primitive erythrocytes begin to circulate throughout the embryo. These cells will soon be replaced by a second wave of multilineage hematopoiesis, when the definitive HSC also appears. This second wave successively originates in different mesodermal domains, the yolk sac first, by E8.5, then in the placenta by E9.5 and finally in the aorta-gonad-mesonephros (AGM) area by E10. Later, about E11-E12, the fetal liver becomes the main site for embryonic hematopoiesis. The HSC and the hematopoietic activity finally move to the bone marrow shortly before birth [4]. Consequently, to this multi-stage and multi-site character of the embryonic hematopoiesis, we will describe how signaling by RA is specifically involved in three main periods, yolk sac and placenta (before E10), AGM (between E10 and E11.5) and fetal liver (after E11.5) (Figure 1).

Figure 1. Main steps of the embryonic and adult hematopoiesis in which the retinoic acid signaling system is involved.



3.1. Early Hematoposiesis

Retinoic acid signaling is involved in both endothelial differentiation and also in the transition from hemogenic endothelium to undifferentiated HSC. Lack of Raldh2 gene expression generates a RA-deficient mutant that dies by embryonic day 10.5 (E10.5) with multiple defects, including lack of organized extraembryonic vessels in the yolk sac [9,28–30] and a strong reduction of hemogenic endothelium (able to differentiate into HSC, see below) and hematopoietic progenitors in the yolk sac, not due to apoptosis. The embryonic endothelium of the yolk sac expresses RARα (both isoforms, 1 and 2), and this expression is prominent (more than 80%) in cells Flk1+/c-Kit+/CD45− which represent the hemogenic endothelium [30]. In these Raldh2-deficient embryos, a number of hematopoiesis-related genes, such as GATA1/2, Scl/Tal1, Lmo2 and Runx1 are much decreased. These developmental defects in the Raldh2−/− mutants can be rescued with exogenous RA treatment *in vivo* and in embryo culture [28,30].

Thus, RA signaling appears to be essential for expression of hematopoietic genes in the earliest hemogenic endothelial cells of the yolk sac. However, detailed analysis of AGM hematopoiesis is not possible in RALHD2 knockout embryos as they die between E9.0 and E10.5 before emergence of the definitive HSC in the AGM [9].

Deficiency of vitamin A in quail embryos allows for initiation of primitive erythropoiesis, but these cells fail to proliferate and survive, leading to severe anemia. The hematopoietic factor GATA2 and the growth factor BMP4 become downregulated in this model. Probably, a lack of BMP4 signaling induces apoptosis in erythroid progenitors [31].

The primitive wave of erythroid differentiation in zebrafish seems also to be regulated by RA, but in an inhibitory fashion. These authors have shown that exogenous RA treatment represses GATA1 expression in zebrafish embryos, leading to a blockade in erythroid differentiation. This effect of RA can be rescued by forced expression of the transcription factor Scl/Tal1. At the same time, RA induced upregulation of Fli1, a vascular marker. Thus, RA is controlling the mesodermal patterning and the balance between vascular and blood cell formation in the early zebrafish embryo, inhibiting erythropoiesis but not vasculogenesis. Cdx4 is located upstream of RA in this pathway, as demonstrated by upregulation of GATA1 expression in Cdx4−/− mutants, and the factor Scl/Tal1, essential for

hematopoiesis, is located downstream RA signaling. The existence of a similar function of RA in primitive erythropoiesis in mammals is suggested by these authors due to the inhibition of the process in cultured mouse yolk sacs or ESC treated with RA. Consequently, treatment of the cultures with the RALDH inhibitor DEAB increased erythroid differentiation [32].

In mouse, Scl/Tal is essential for both establishing the hematopoietic transcriptional program in hemogenic endothelium and preventing its misspecification to a cardiomyogenic fate [33]. Recent data suggest that endocardial/endothelial cells expressing cardiac markers serve as a *de novo* source for transient definitive hemopoietic progenitors [34].

On the other hand, Goldie *et al.* (2008) reported normal amounts of Ter119+ and CD41+ cells in RALDH2-knockout embryos of 6–8 somites (about stage E8.0). Thus, RA could just be first playing a modulating role in the formation of the primitive erythrocytes and endothelial cells of the yolk sac and then an essential role in the induction of the hematopoietic genes in a subpopulation of endothelial cells that become the hemogenic endothelium, as described in the next section [30].

3.2. Hematopoiesis in the AGM Region

Definitive HSC originate from the hemogenic endothelium [35,36]. This kind of embryonic endothelium, transiently localized in some vessels such as the aorta, the vitelline and the umbilical arteries, is able to give rise to short term and long term hematopoietic stem cells in the process known as endothelial-hematopoietic transition (EHT) [6]. In fact, most of the definitive HSC emerge from the hemogenic endothelium of the aorta in the aorta-gonad-mesonephros (AGM) region of the embryo between E10.5 and E11.5 in the mouse embryo. These definitive HSC expand in the fetal liver before homing in the bone marrow, the site of definitive hematopoiesis in mammals [36].

A recent paper has significantly clarified the role played by RA signaling in AGM demonstrating that this signaling system is essential for HSC emergence from the hemogenic endothelium of the aorta Activation of RA signaling pathway in isolated hemogenic endothelium or pre-hematopoietic stem cells (as identified by colocalization of endothelial and hematopoietic genes) enhances generation of HSCs. On the other hand, conditional deletion of RALDH2 in endothelium (using a VE-Cadherin-Cre/ Raldh2 flox system) inhibits HSC development in both, yolk sac and aorta, demonstrating that the endothelium itself is the source of RA. These authors also demonstrated that the RA signal for the endothelial-hematopoietic transition is transduced through RARα, as expected by the wide expression of this receptor in the endothelium. However, the lack of this receptor can be compensated by other RA receptors, as suggested by the lack of a similar phenotype in RARα knockout mice as described above. Since it was known that canonical Wnt signaling and β-Catenin degradation is required for the EHT. Chanda *et al.* demonstrated that RA treatment of the hemogenic endothelium led to inhibition of Wnt signaling and β-catenin degradation, thus mechanistically connecting the RA and the Wnt/β-catenin pathway in the EHT [37]. Also, has been demonstrate that Wnt that β-catenin activity is needed for the emergence but not the maintenance of HSCs in mouse embryos [38].

The stem cell factor receptor c-Kit, expressed by the hemogenic endothelium, seems to be also critically involved in the signaling pathway dowstream of RA. RALDH2 deficient mouse embryos show downregulation of this receptor. Reexpression of c-Kit in RALDH2-deficient endothelium rescue the hemogenic phenotype and particularly increases expression of p27, regulating the cell cycle, and

Notch1, required for arterial specification of the endothelium. Reexpression of p27 in Raldh2-deficient, Notch1 inactivated endothelial cells, seems to be enough to rescue their hemogenic specification. The hematopoietic gene Runx1 is upregulated downstream of c-Kit. Thus, c-Kit and Notch1 are located downstream of RA signaling and p27 in the specification of the hemogenic endothelium, whereas Runx1 regulates the generation and/or propagation of multilineage HSC from the hemogenic endothelial cells [39,40].

3.3. Hematopoiesis in the Fetal Liver

In the fetal liver stage, RA also plays a role in hematopoiesis, particularly in the differentiation of erythrocytes. Erythropoietin (EPO) is an essential factor for definitive erythropoiesis. The early wave of erythropoiesis in the yolk sac, occurring in mice between E7.5 and E9.5 is EPO independent. In contrast, the early phase of fetal liver erythropoiesis requires EPO, retinoic acid signaling through RAR/RXR receptors and also stabilization of the hypoxia inducible factor (HIF1). The EPO promoter has a type DR2 RARE (retinoic acid response element) located very close to a HIF1 binding site which promotes EPO expression in hypoxia. Interestingly, RXRα requirement is only active during this early phase (E9.5-E11.5), as erythropoiesis can continue without RXRα signaling from E12 on. In fact, EPO expression is 10 fold lower in RXRα −/− fetal liver as compared with wildtypes by E10, but there are no differences by E12, suggesting that the RXRα receptor has become dispensable for activation of the EPO promoter. Consequently, other elements of the retinoic signaling pathway, such as RALDH1/2, CRBP1, CRABP1/2 are highly expressed in the early fetal liver, becoming downregulated by midgestation. Thus, RA signaling is a key factor for initiation of fetal liver erythropoiesis [41].

In summary, RA signaling is critically required in multiple stages of developmental hematopoiesis, particularly in the specification of the hemogenic endothelium, and also during the early stage of erythropoiesis in the fetal liver (Figure 1).

4. RA and Adult Hematopoiesis

The receptors RARα and γ are highly expressed in the adult hematopoietic system. The RARβ receptor shows a low level of expression and in fact seems not to be involved in adult hematopoiesis. Since loss of function of RARα and γ results in viable mice as described above, these murine models allow for study of the modulatory signaling role played by retinoic acid in adult hematopoiesis. This role would be supported by epidemiological studies pointing to an association of vitamin A deficiency with anemia in humans [24].

RARα is expressed in a large range of cells of the bone marrow, while RARγ is expressed in primitive HSC. RARγ −/− mice in particular show a defect in the number of bone marrow HSC [22], they are anemic [24] and develop myeloproliferative syndrome, although the latter defect is not due to the function of RARγ in the HSC themselves, but in the cellular niche of the bone marrow [23]. The anemic phenotype seems also to be due to the RARγ function in cells other than erythroid progenitors. When RARα, RARγ and both receptors are conditionally deleted in cells expressing the EPO receptor (EpoR), adult mice display normal hematopoiesis [24].

The Notch pathway in HSC is particularly affected by the loss of function of the RARγ, since Notch1 and its effector Hes1 are downregulated in mutant mice while Hoxb4 expression is

normal [22]. Notch and Hoxb4 are regulators of HSC self-renewal [42,43]. On the other hand, RARγ is dispensable for lymphopoiesis, although mice lacking this receptor display defective primary and memory CD8+ T cell response, and their macrophages show impaired inflammatory cytokine production [44].

Thus, RA signaling transduced through RARγ receptor is involved in HSC self-renewal and repopulation potential. In contrast, RARα −/− mice show no defects in the early steps of adult hematopoiesis and it is not involved in the self-renewal potential of HSC, although signaling transduced through RARα is critically involved in granulocytic lineage modulation, as described below.

In humans, the RA/RAR signaling system also plays a key role in the balance between self-renewal and differentiation established in the bone marrow microenvironment. This role could have interesting clinical applications. Pharmacological inhibition of aldehyde dehydrogenases with DEAB allows for expansion of HSC in culture, preventing their differentiation. Inhibition of RA signaling induces upregulation of Hoxb4, keeping HSCs in an undifferentiated stage. This effect is cancelled by treatment with retinoids and vitamin D [45]. On the other hand, primitive CD34+/CD38− HSC express aldehyde dehydrogenase 1 and RARα [46]. These primitive HSC differentiate when cultured *ex vivo*, but inhibition of the RA signaling pathway in culture keeps these cells in a primitive state, allowing for their expansion. RA degradation by the enzyme CYP26 from stroma could be involved in this mechanism, contributing to the maintenance of the pool of self renewing HSCs. This study suggests a role for RARα in human HSC different to that described in mice.

The differentiation along the granulocyte lineage is modulated by RA through the RARα receptor. Free RARα receptor, not bound to their ligands, inhibits neutrophile differentiation while RA bound to this receptor induces neutrophil differentiation [20]. Overexpression of RARα induced by retroviral transduction increases the number of granulocytes while overexpression of RARγ induces increase of undifferentiated progenitors [22]. A similar effect is observed in mice treated with vitamin A deficient diet or with RAR antagonists. In these mice, an increase of myeloid cells is observed in bone marrow, spleen and peripheral blood [47,48]. The expansion of myeloid cells leads to a severe splenomegaly in 14 weeks old mice receiving a vitamin A deficient diet from birth. This condition can be partially reverted by a vitamin A supplemented diet. It is important to remark that this effect of vitamin A deprivation has not incidence on the number of colony forming units obtained from the bone marrow. Thus, the frequency of myeloid progenitors is not influenced by the lack of RA. Instead, frequency of apoptosis in CD11b+/Ly-6G+ cells is significantly lower in vitamin A deprivated mice [47].

The role played by RARα is related with the pathogeny of acute promyelocytic leukemia (APL). This disease is produced by a chromosomal rearrangement fusing the RARα gene with other genes such as PML and PLZF and generating a dominant negative form of RARα. Thus, lack of function of the receptor results in undifferentiated promyelocytic progenitors that cause the leukemia [49]. The key role played by the receptor in this disease is emphasized by the remission observed in 75% of the APL cases after treatment with ATRA [50–53].

Besides these functions of RARs in normal and pathological hematopoiesis, it is important to emphasize that RXRα is an important heterodimerization partner of RARs and it is normally required for RA signaling in all the above described processes. RXRα seems to be dispensable for adult hematopoiesis [16]. However, RXRα might be necessary for the pathogenesis of APL [54].

5. Vitamin D and Hematopoiesis

Physiological functions mediated by Vitamin D are closely related to retinoid signaling. The vitamin D receptor VDR can form a homodimer or a heterodimer with a retinoid X receptor, binding to vitamin D response elements (VDREs) and eventually leading to target gene transcription [55].

Mice whose VDR has been genetically deleted have normal numbers of granulocytes, monocytes, lymphocytes, platelets and erithrocytes [56,57]. However, other results suggest that VDR loss in the hematopoietic environment results in increased splenic residence of HSCs, possibly the result of dysregulation of extracellular calcium [58]. Also, VDR may have a potential benefit in controlling homing from BM to improve the clinical efficiency of stem cell transplantation [59].

On the other hand, vitamin D seems to modulate in some way hematopoiesis since treatment of hematopoietic stem cells or some lines of leukemia cells with the active form of this molecule leads to increased monocyte/macrophage differentiation, an effect which is not detected in VDR knock-out mice [59,60]. Interestingly, RAR receptor activation induces granulopoiesis, as described above. It is possible that VDR and RAR compete for RXR heterodimerization. In this way, VDR/RXR and RAR/RXR heterodimers would drive differentiation of progenitors towards monopoiesis and granulopoiesis respectively [60,61].

6. Potentiating Factors for RA Signaling in Hematopoiesis

A number of mechanisms can modulate RA signaling in the normal and pathological hematopoietic processes above described. For example, the differentiating role of RA on the promyelocytic leukemia HL-60 cell line is enhanced by prostaglandin-E2 and cholera toxin [62] agents which increase the intracellular level of cAMP, an intracellular signal involved in APL cell differentiation. Cytokines such as GM-CSF, G-CSF, IL-3 and IL-1 also modulate the effect of RA on APL cells [63].

The signal transducer and activator of transcription 5 (Stat5), a key element of the interleukin/Jak receptor signaling system, physically interacts with RARs in a IL3 dependent manner enhancing RAR transcriptional activity [64], thus opening the possibility of a cross-talking between the Jak/Stat and the retinoid pathways in different stages of hematopoiesis. In fact, IL-3 and GM-CSF regulate the activity of RARs in models of myeloid differentiation *in vitro* [65,66].

7. Concluding Remarks and Future Directions

The retinoic acid signaling pathway is involved in many developmental processes, and in the last years its key role in the multiple steps of developmental and adult hematopoiesis has also been demonstrated. A better knowledge of the upstream regulators of this important signaling system and their genetic targets would give us a much clearer perspective of the mechanisms leading to the differentiation of the blood cells during development and, even more important, the fine regulation of the blood cell lineages in adults. This knowledge is essential since anomalies in the mechanisms participating in this regulation are the cause of multiple pathologies of the blood system. Furthermore, *in vitro* expansion and differentiation of the human hematopoietic stem cells would be a great clinical achievement for the treatment of all type of anaemias. As shown in this review, retinoic acid signaling is suggested to be a protagonist for both future goals.

Table 1. Summary of the hematopoietic phenotypes derived from genetic or dietary alterations of the elements of the retinoic acid (RA) signaling system.

		Knock out	Phenotype	References
RAR	α	**RARα −/−**	Early lethality (lifespan usually < 2 months). Accelerated granulocytic differentiation of bone marrow in culture. No defects in bone marrow or erythropoiesis *in vivo*.	[17,20,22,24,67,68]
		RARα1 −/−	Normal phenotype.	[17,18]
	β	**RARβ −/−**	Growth deficiency and several defects, although no apparent hematopoietic abnormalities.	[21,69,70]
		RARβ2 −/−	Normal phenotype.	[69]
		RARβ1 −/− **RARβ3 −/−**	Normal phenotype.	[71]
	γ	**RARγ −/−**	Early lethality (<40% survival by 3 months). Increased granulocyte/macrophage progenitors in bone marrow. Chronic myeloproliferative syndrome. Reduced erythropoiesis. Phenotype probably caused by RARϒ function in stroma.	[19,22–24,72]
		RARγ1 −/−	Growth deficiency and several defects, although no apparent hematopoietic abnormalities.	[73]
		RARγ2 −/−	Normal phenotype.	[19,73]
	Double mutants	**RARα −/−** **RARγ −/−**	Embryonic lethality, although no apparent hematopoietic abnormalities.	[19,20,74]
		RARα1 −/− **RARγ −/−**	Postnatal lethality. E18.5 bone marrow cells show a blockage of myelocitic differentiation.	[25]
		Conditional deletion of RARα, RARγ and both receptors in EPO+ cells	Normal erythropoiesis.	[24]
		RARα −/− **RARγ1 −/−**	No additional defects to those described for RXRα /RXRγ -/-.	[73,74]
		RARα −/− **RARγ2 −/−**	No additional defects to those described for RXRα /RXRγ -/-.	[73]
		RARβ2 −/− **RARγ −/−**	Ocular defects. No apparent hematopoietic abnormalities.	[74,75]
		RARα −/− **RARβ2 −/−**	Several defects, although no apparent hematopoietic abnormalities.	[74]

Table 1. *Cont.*

		Knock out	Phenotype	References
RXR	α	**RXRα −/−**	Die at E13.5-E16.5 with severe cardiac defects. Fetal liver erythropoiesis is transiently compromised prior E12.5.	[14,15,39,65]
		Conditional deletion of RXRα −/− in HSC	Normal phenotype. *In vitro*, RXRα −/− bone marrow cells form colonies more efficiently than those from control mice in the absence of ligand. This colony formation capacity is suppressed by the addition of 9cis-RA.	[16]
	β	**RXRβ −/−**	50% of mutants died during gestation or at birth, the other half are normal except for an abnormal spermatogenesis in males.	[12,13]
	γ	**RXRγ −/−**	Normal phenotype.	[12]
	Double mutants	**RXRα −/− RXRϒ −/−**	Growth deficiency and several defects, although no apparent hematopoietic abnormalities.	[12]
		RXRβ −/− RXRγ −/−	No additional defects to those described for RXRα /RXRβ −/−.	[12]
		RXRα −/− RXRβ −/− RXRϒ −/−	Growth deficiency and male sterility, no apparent hematopoietic abnormalities.	[12]
RALDH	2	**RALDH2 −/−**	Die at E10.5 with multiple anomalies. Vascular defects due to an abnormal endothelial cell development. Hematopoietic defects not described.	[9,28–30,37]
		Conditional deletion of RALDH2 in VE-cadherin expressing cells	Defective yolk sac hematopoiesis. No long-term HSC formation in AGM (no reconstitution when transplanted into irradiated mice).	[35]
	3	**RALDH3 −/−**	Die at birth because respiratory failure. No apparent hematopoietic abnormalities.	[8]
Vitamin A deficiency		**Mice fed a vitamin A deficient diet**	Expansion of myeloid cells in bone marrow, spleen and peripheral blood. No changes in CFU potential.	[45]
		CRBPI −/−	Low vitamin A store. When fed with vitamin A deficient diet, animals develop an expansion of neutrophils in spleen and peripheral blood with an increase of relatively immature granulocytes.	[20]

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Author Contributions

EC has written about adult hematopoiesis and prepared the Table 1. LA has written about primitive hematopoiesis. RMC has written about adult hematopoiesis and Vitamin D and hematopoiesis, and has designed the figure. RC has written about embryonic hematopoiesis.

Conflicts of Interest

The authors declare no conflict of interest.

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