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Review

Evolutionary Origin of the Proepicardium

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Abstract: The embryonic epicardium and the cardiac mesenchyme derived from it are critical to heart development. The embryonic epicardium arises from an extracardiac progenitor tissue called the proepicardium, a proliferation of coelomic cells located at the limit between the liver and the sinus venosus. A proepicardium has not been described in invertebrates, and the evolutionary origin of this structure in vertebrates is unknown. We herein suggest that the proepicardium might be regarded as an evolutionary derivative from an ancient pronephric external glomerulus that has lost its excretory role. In fact, we previously described that the epicardium arises by cell migration from the primordia of the right pronephric external glomerulus in a representative of the most primitive vertebrate lineage, the lamprey *Petromyzon marinus*. In this review, we emphasize the striking similarities between the gene expression profiles of the proepicardium and the developing kidneys, as well as the parallelisms in the signaling mechanisms involved in both cases. We show some preliminary evidence about the existence of an inhibitory mechanism blocking glomerular differentiation in the proepicardium. We speculate as to the possibility that this developmental link between heart and kidney can be revealing a phylogenetically deeper association, supported by the existence of a heart-kidney complex in Hemichordates. Finally, we suggest that primitive hematopoiesis could be related with this heart-kidney complex, thus accounting for the current anatomical association of the hematopoietic stem cells with an aorta-gonad-mesonephros area. In summary, we think that our hypothesis can provide new perspectives on the evolutionary origin of the vertebrate heart.

Keywords: epicardium; evolution; development; pronephros; glomerulus

1. Introduction

The prevailing view maintained during two thirds of the 20th century about the origin of the epicardium through differentiation of the outer layer of the cardiac wall (the so-called "epimyocardium") was challenged by the seminal work by Manasek [1,2] who confirmed previous and lengthily neglected studies of His [3] and Kurkiewicz [4]. These works described the origin of the epicardium from an extracardiac cluster of cells, first called "pericardial villi" and currently known as the proepicardium. Thus, the supposed "epimyocardium" was rightly recognized as exclusively constituted of myocardial cells.

The proepicardium appears on the coelomic wall, just behind the caudal limit of the heart. The proepicardium is frequently a single structure located at the right side in most vertebrates, although it usually arises from bilaterally paired primordia (reviewed in [5]). Proepicardial cells attach to the heart surface, either directly or after they are released into the coelomic cavity. Then, proepicardial cells spread over the cardiac surface to give rise to the epicardium.

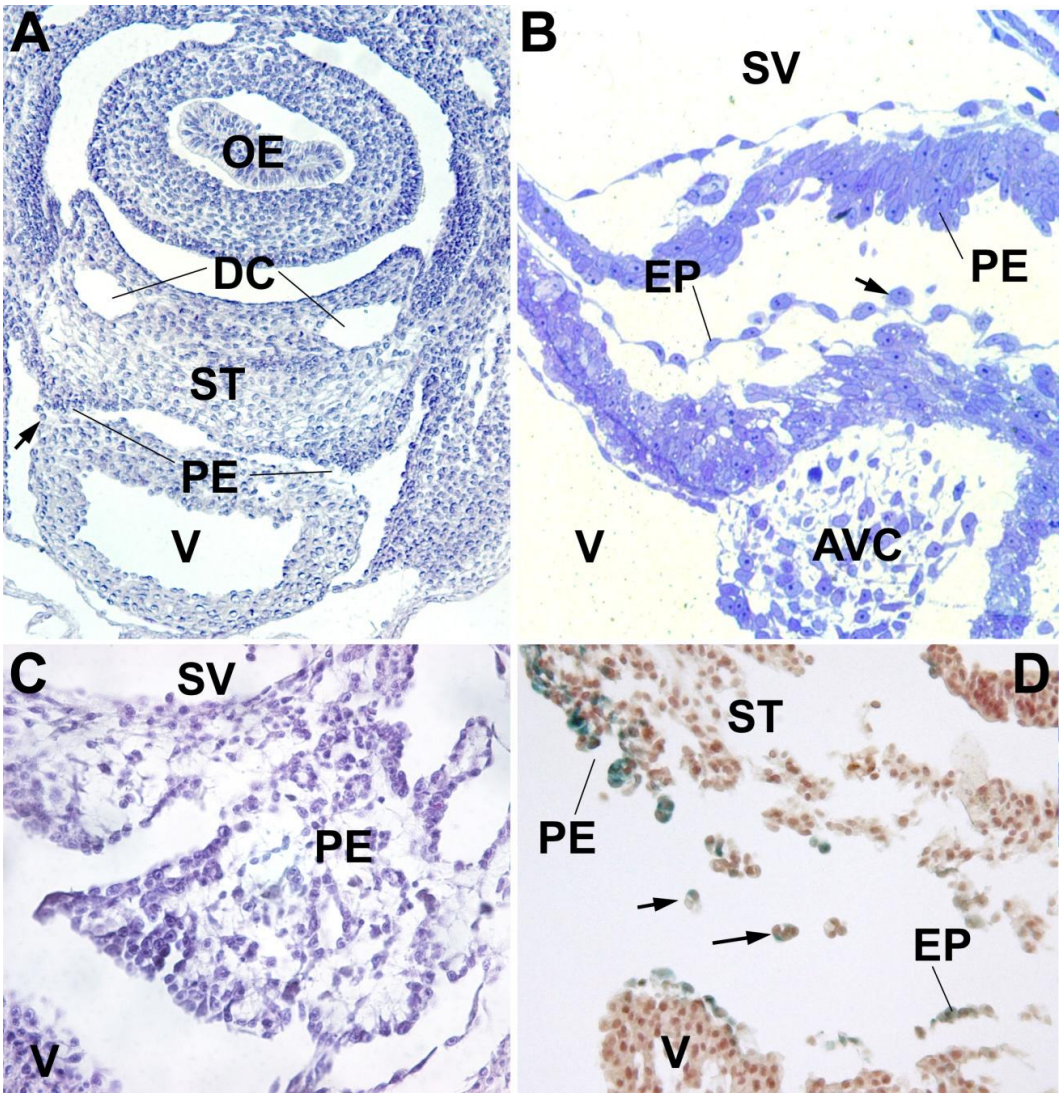
The increasing attention that the embryonic epicardium has received as an essential element for cardiac development, actively interacting with the developing myocardium, has encouraged interest in the peculiar way in which the proepicardium appears. Although some non-vertebrate hearts also are lined by an epicardium (e.g., molluscs [6]), the investment of the embryonic heart by an extracardiac primordium seems to be exclusive of (and generalized to all) the vertebrates. This particularity and the peculiar gene expression profile of the proepicardium and the epicardial cells (including a number of kidney-related genes) have raised questions about the evolutionary origin of the proepicardium. We have forwarded a hypothesis about the origin of the proepicardium as an evolutionary remain of a pronephric external glomerulus [7]. We think that this hypothesis can explain most of the peculiarities of the epicardial development and also it relates the early cardiac evolution in vertebrates with the excretory and hematopoietic systems rather than with the branchial gas-exchange system as it has been traditionally assumed. We herein review the evidence supporting this hypothesis about the evolution of the proepicardium.

2. Comparative Anatomy of the Proepicardium

With the exception of the epicardial development in lampreys, which will be described in the next section, the epicardium of all the vertebrates derives from two clusters of cells located slightly over the caudal limit of the sinus venosus (Figure 1) (reviewed in [5]). The fate of these primordia differs among vertebrate species. A single proepicardium arises from the asymmetric development of the right primordium in amphibian, reptiles and birds [8,9]. Mammals show symmetric development of the primordia giving a medial proepicardium [10]. In contrast, paired proepicardia appear in the dogfish *Scyliorhinus*, an elasmobranch [10–12], although only the right one contacts with the heart surface [7] (Figure 1A,B). Among bony fishes, the sturgeon also has a bilaterally symmetric proepicardial development [13], although the proepicardium is single and medially located in the zebrafish [14].

Depending on the development of other structures located at the level of the proepicardium, it can lie over the liver wall, the septum transversum, or ventrally to the right ductus of Cuvier and the right horn of the sinus venosus.

Figure 1. Comparative anatomy of the proepicardium. **(A,B)** In the dogfish (*Scyliorhinus canicula*), the proepicardium (PE) is paired, and it is located over the septum transversum (ST) in its caudal part and over the sinus venosus (SV) in its cranial part. Only the right proepicardium contacts the heart surface, as shown by the arrow in A. A detail of the left proepicardium is shown in B. The epicardium (EP) is already formed by this stage (23 mm total length) and some floating cells can be adhered to the epicardial surface (arrow in B). AVC: atrioventricular endocardial cushion; DC: ducti of Cuvier; OE: oesophagous. V: ventricle. **(C)** Chick proepicardium, stage HH17. The proepicardium (PE) is large, contains abundant mesenchymal cells and extracellular matrix and it is located below the sinus venosus (SV). **(D)** Mouse proepicardium, stage E9.5. This embryo expresses the reporter LacZ under control of the Wt1 promoter. Wt1 expressing cells are observed in the proepicardium (PE), located over the septum transversum, the epicardium (EP) and also in free floating groups of cells (arrows).



The proepicardium can release free floating cells or small cellular vesicles into the pericardial cavity, cells that finally adhere to the myocardial surface. This has been described in most species studied including the dogfish [12], the sturgeon [13], the amphibian *Ambystoma mexicanum* [15], the chick [16], the mouse [17] and the tupaia [18] (Figure 1D). In the chick embryo, this mechanism has little importance [19,20]. Independently of the releasing of free floating cells, in all cases, the proepicardium finally attaches to the myocardium of the dorsal surface of the ventricle and the inner curvature of the heart, sometimes forming a firm tissue bridge [8,15] and spreading later throughout the cardiac surface. The whole proepicardium is usually transferred in this way to the heart surface. However, in *Scyliorhinus*, the paired late proepicardial remains show signs of apoptosis and disappear [11,12]. In some amphibians and reptiles, the proepicardial attachment site remains, forming the so-called sinu-ventricular ligament, a structure independent of the pericardial stalk located at the ventricular apex in many reptiles [21–23].

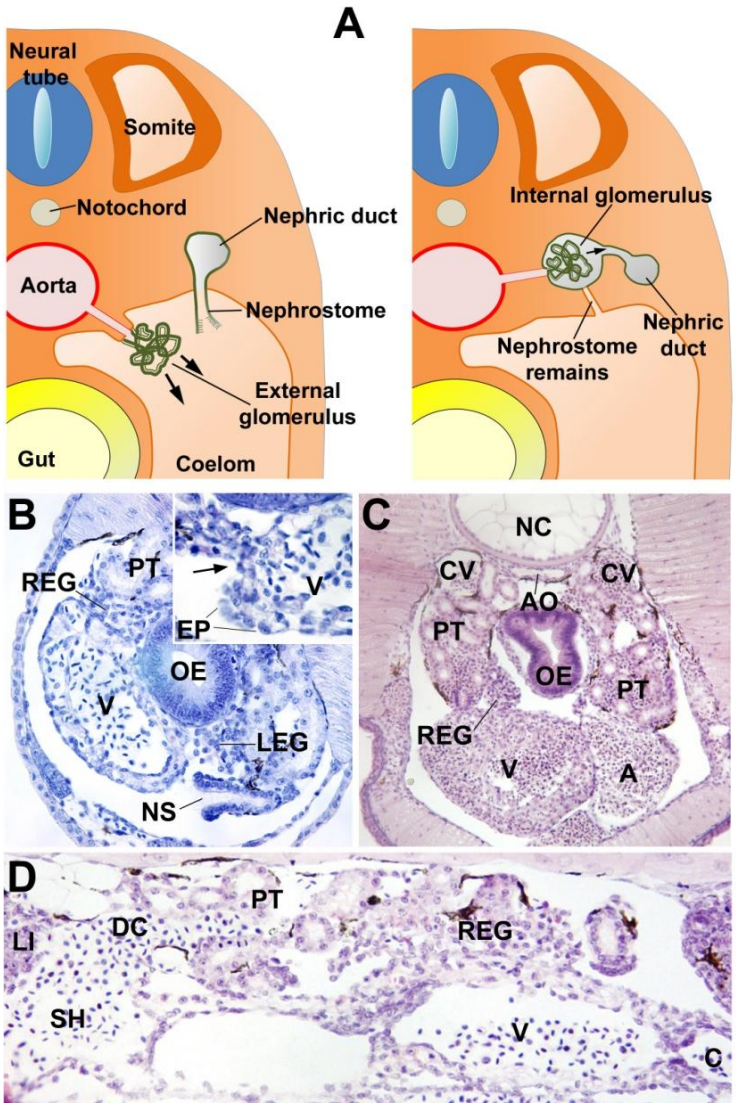
The avian proepicardium is composed of large villi containing abundant mesenchymal cells and extracellular matrix (Figure 1C). These villi appear in the chick embryo by the stages HH15-16. The proepicardium decreases in size as the epicardium spreads on the cardiac surface, and it completely disappears by the stage HH25-26 [24]. In the mouse embryo, the proepicardium appears on the surface of the transverse septum by 9 dpc, it is smaller than that of the avian embryos, and it is constituted of clusters of rounded cells, first, and later of mesothelial villi and mesenchymal cells [17,25] (Figure 1D).

3. The Proepicardium as the Evolutionary Remain of a Pronephric External Glomerulus

The basic structure of the primitive excretory system of vertebrates was composed of (1) A set of external glomeruli, vascular clusters connected to the aorta, protruding into the coelomic cavity, and surrounded by podocytes acting as blood filters, and (2) Ciliated nephrostomes aspirating the filtrate from the coelomic cavity and a system of collecting tubules to drain the filtrate out of the body. This primitive organization based in two independent compartments became merged later when glomeruli formed within the body wall and they were surrounded by cavities connected to the collecting tubules (Figure 2). In this way, the general coelomic cavity was excluded from the excretory system. However, in some cases this primitive organization of the excretory system can still be observed in larval stages of some vertebrates. This is the case of some amphibian tadpoles [26,27]. In chick embryos, external glomeruli transiently form but they degenerate and probably they do not perform any excretory function [28].

The lampreys, together with the hagfishes (myxines) are representatives of the Agnathans, the phylogenetically most primitive lineage of extant vertebrates. Lamprey larvae also develop paired pronephric external glomeruli in the cranial part of the coelom, over the heart. Interestingly, the right embryonic primordium of these glomeruli acts like a proepicardium, contacting the cardiac ventricular surface and giving rise to cells that spread over the heart and constitute the epicardium (Figure 2). Later this "proepicardium" continues its differentiation and gives rise to a fully functional external glomerulus [7].

Figure 2. External glomeruli and epicardial development in lampreys (*Petromyzon marinus*). **(A)** The primitive organization of the vertebrate excretory system is shown in the left panel. An external glomerulus filters blood from the aorta and releases the filtrate (arrows) into the coelomic cavity, where it is aspirated by ciliated nephrostomes and drained through nephric ducts. The advanced condition is depicted in the right panel; the glomerulus has become isolated into an internal cavity connected to the nephric ducts. The remains of the nephrostomes are sometimes visible in some cases. **(B-D)** Origin of epicardial cells from the attachment of the right external glomerulus (REG) to the ventricle (V) in embryos and larvae of lampreys. The attachment is shown in an embryo of 23 dpf (B) and a larva of 15 mm (C). The insert in B shows a higher magnification view of the site of attachment (arrow) and the early epicardial (EP) cells on the ventricle in the 23 dpf embryo. The left external glomerulus (LEG) and the nephrostome (NS) are shown in B. Note the position of the ventricle, at the right side, and the atrium (A) at the left. In D, the association of the right external glomerulus to the ventricle is shown in a sagittal section of a 26 dpf embryo. Note the large distance between the glomerulus and the liver (LI). AO: aorta; C: conus arteriosus; CV: precardinal veins; DC: ducts of Cuvier; OE: oesophagous; PT: pronephric tubules; SH: suprahepatic vein.



This observation moved us to propose that the proepicardium of all the other vertebrates is a remnant of the ancestral right pronephric external glomerulus which lose its filtrating function but remains to provide not only the epicardial lining of the heart but also vasculogenic cells for the primitive coronary plexus (Figure 3). Epicardial-derived cells have proven to be essential for the early cardiac vascularization and the development of the ventricular compact layer [29–32]. Interestingly, the asymmetric contribution to the epicardium, from cells migrating from the right pronephric glomerulus, probably due to the primitive position of the cardiac ventricle at the right side (see Figure 2B,C), has been maintained in the evolution. As stated above, the proepicardium is a right-sided structure or, when paired, as in the case of elasmobranchs, only the right proepicardium contacts the heart surface.

Alternatively, it can be argued that epicardial development in lampreys is a derived (apomorphic) condition, and that the proepicardium is an evolutionarily new structure developed by jawed vertebrates. However, we think that the peculiar gene expression profile of the proepicardium, as described in the next section, does not support this alternative explanation.

A criticism that could be raised against our proposal is that other vertebrates that develop pronephric glomeruli, such as zebrafish or *Xenopus* larvae, also develop an independent proepicardium, which is not related to these glomeruli. However, it is important to remark that the pronephros in these species appears in a relatively caudal location respect to the heart, between somites 3 and 5 in amphibians [27] and at the level of the 3rd somite in zebrafish [33]. The uncoupling of the cardiac and the pronephric domains, which are still anatomically associated in agnathans, probably occurred during the transition to jawed vertebrates, leaving the embryonic heart-associated, most cranial glomerular primordium, devoid of its excretory potential, ahead of the pronephric domain (Figure 3).

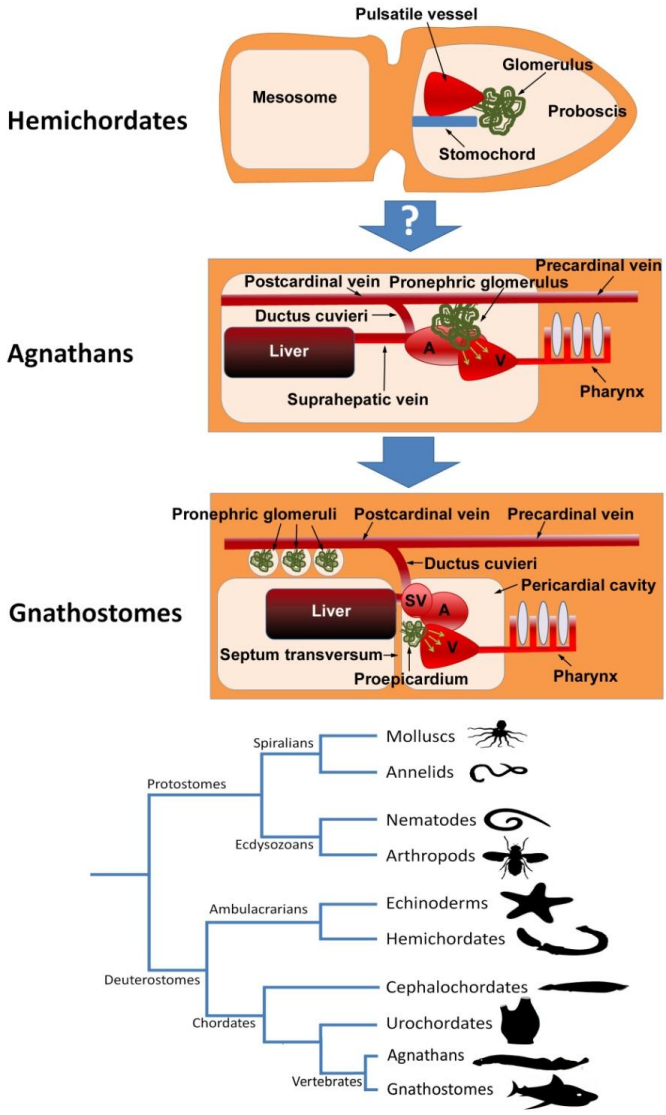
4. Gene Expression in the Proepicardium. Comparison with Kidney Development

The expression of a number of genes has been demonstrated to occur (and some times to be required) in both, epicardial and kidney progenitors. Wt1, the Wilms' tumor suppressor gene, is expressed in specific areas of the coelomic epithelium and also in mesenchymal cells (probably derived from the coelomic epithelium in most cases) of many visceral organs. Wt1 is essential for kidney development (in fact, Wt1 expression continues in adult podocytes) and also for the differentiation of epicardial-derived cells [34]. Conditional deletion of Wt1 in epicardium leads to a failure in epicardial epithelial-mesenchymal transition and to defective coronary artery development [32].

The bHLH transcription factor Tcf21/capsulin/epicardin/Pod1 is expressed in podocytes, epicardial and epicardial-derived cells as well as in other organs. Tcf21 knockout mice show defects in glomerular development [35,36]. Interestingly, Tcf21seems to be dispensable for epicardial development but it is required for fibroblastic differentiation of epicardial-derived cells [37]. The T-box factor Tbx18 is also expressed in the venous pole of the heart, the proepicardium and the developing epicardium [38]. Tbx18 is essential for kidney development and it is considered as dispensable for epicardial development, although a recent report relates Tbx18 with Snail2 regulation and epithelial-mesenchymal transition control in epicardial cells [39]. Nephrin (NPHS1) a major structural protein of kidney podocytes, reported as essential for proper podocyte function [40], is also expressed in the epicardial cells and its loss of function leads to abnormal development of epicardium

and coronary vessels [41]. Nestin, an intermediate filament protein dynamically expressed by a variety of progenitor cells during development, is strongly expressed in podocytes and also in the embryonic epicardium and coronary vessels. In both cases, the expression of this protein seems to be regulated by Wt1 [42]. Finally, we have found by RT-PCR expression of Glepp1, the glomerular epithelial protein-1, in the chick proepicardium (unpublished observations). Glepp1 is a membrane protein-tyrosine phosphatase essential for podocyte structure and function [43].

Figure 3. Hypothetical evolution of the proepicardium from an external pronephric glomerulus. The development of the pronephros at a more caudal level in gnathostomes leaves the most cranial pronephric glomerulus associated to the heart and devoid of its original excretory function and consequently of its connection with the aorta. The position of the proepicardium changes due to the development of a sinus venosus (ill-defined in agnathans), the most ventral position of the ventricle respect to the atrium, the development of a septum transversum and the approaching of the liver. A potential relationship of the agnathan pronephros/heart association with the heart/kidney complex of hemichordates is also suggested. The tree shows a much simplified diagram of the phylogenetic relationship of these and other phyla mentioned in the text.



Besides coincidence in gene expression profiles, proepicardium and pronephric glomerulus show similar signaling pathways responsible for their induction. Retinoic acid (RA) and activin are main inducers of the pronephric glomus and tubules in *Xenopus* [44]. This is in agreement with the role played by retinoic acid and the activin receptor ALK2 in proepicardial and epicardial development [30,31,45]. Proepicardial apoptosis has been described in RXRα-null embryos [46]. Since RA is also an inducer of mouse podocyte differentiation *in vitro* and *in vivo* [47] it can be asked why proepicardial cells responsive to RA signals does not differentiate into podocytes. As we will discuss below we think that this differentiation pathway is actively blocked. Anyhow, we have found evidence of a strong upregulation of podocyte specific markers in chick proepicardial cells after treatment with RA (unpublished observations).

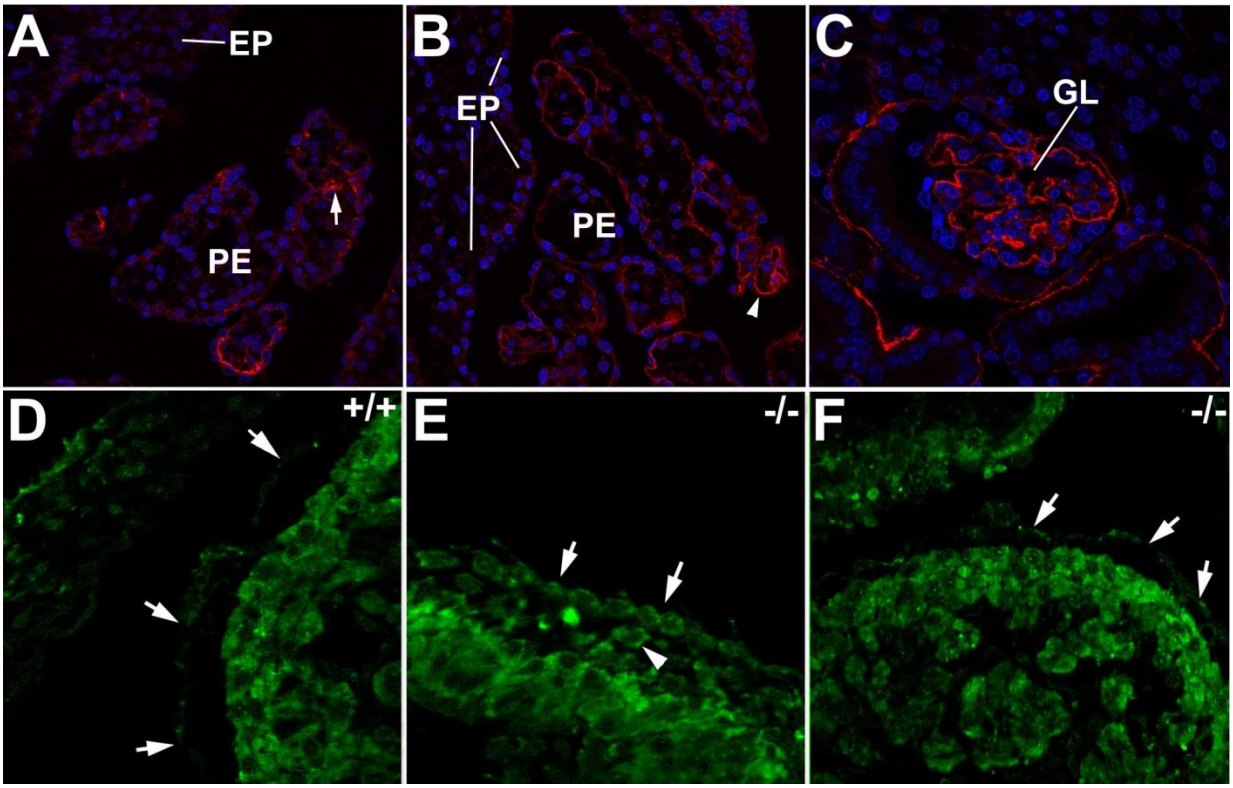
The proepicardium expresses many of the genes required for glomerular differentiation, but it does not fully achieve such a differentiation. It is possible that the myocardium be the source of inhibitory signals to the epicardium to block podocyte differentiation and mesenchymal to epithelial transition, keeping active the program of vascular differentation. In fact, when the proepicardial adhesion to the heart in chick embryos is partially blocked, we can still observe proepicardial villi in late embryos, about HH29, when all the proepicardial villi should have disappeared. Some cells from these villi, especially those located far away from the heart, show an upregulation of laminin, in a similar fashion to that observed in the developing glomerulus and suggesting a mesenchymal-epithelial transition (Figure 4A-C). As a control, the epicardium *in situ* and the EPDC are always laminin-negative by this stage.

The Wilms' tumor suppressor gene Wt1 might be related to the mechanism of repression of the glomerular fate of the proepicardium. A recent study has shown how Wt1 is promoting expression of Wnt4 in the nephrogenic mesenchyme and at the same time, Wt1 represses Wnt4 in the epicardium [48]. Since Wnt4 induces mesenchymal to epithelial transition in the nephrogenic mesenchyme, this mechanism could be a key to explain the repression of glomerular differentiation in the proepicardium. This means that lack of expression of Wt1 in the proepicardium could at least initiate glomerular differentiation in this tissue and in fact, Wnt4 expression is increased in cultured epicardial cells deficient from Wt1 [48]. We have also observed E-cadherin immunoreactive cells in the epicardium and subepicardium of Wt1-null embryos, suggesting a mesenchymal to epithelial transformation of epicardially-derived cells (Figure 4D-F). Thus, the blocking mechanism for glomerular differentiation of the proepicardium can be due to both, an inhibitory signal from the myocardium and a tissue-specific downregulation of Wnt4 by Wt1 (Figure 5).

The paired-box gene Pax2 is, together with Pax8, essential for nephrogenesis, and particularly for the mesenchymal to epithelial transition needed for the early stages of kidney development [49–51]. It is interesting to note that while intermediate mesoderm markers such as Lim2 and Odd1 are expressed throughout the band comprised between the somites and the lateral mesoderm, including the area where the proepicardium arises, Pax2 cranial expression starts at the level of the 4th somite, and it is not expressed in the proepicardium (which always develops cranially to somite 4 in chick and mouse embryos). Since Pax2 expression only occurs in the mesenchyme, which has been induced by a nephrogenic signal [52], it is conceivable that this signal is repressed in the most cranial intermediate and lateral mesoderm, possibly by Wt1 itself [53]. In fact, we have found that Pax2 is expressed in the

chick proepicardium when this tissue is cultured in isolation, thus supporting the existence of such an inhibitory signal (unpublished observations).

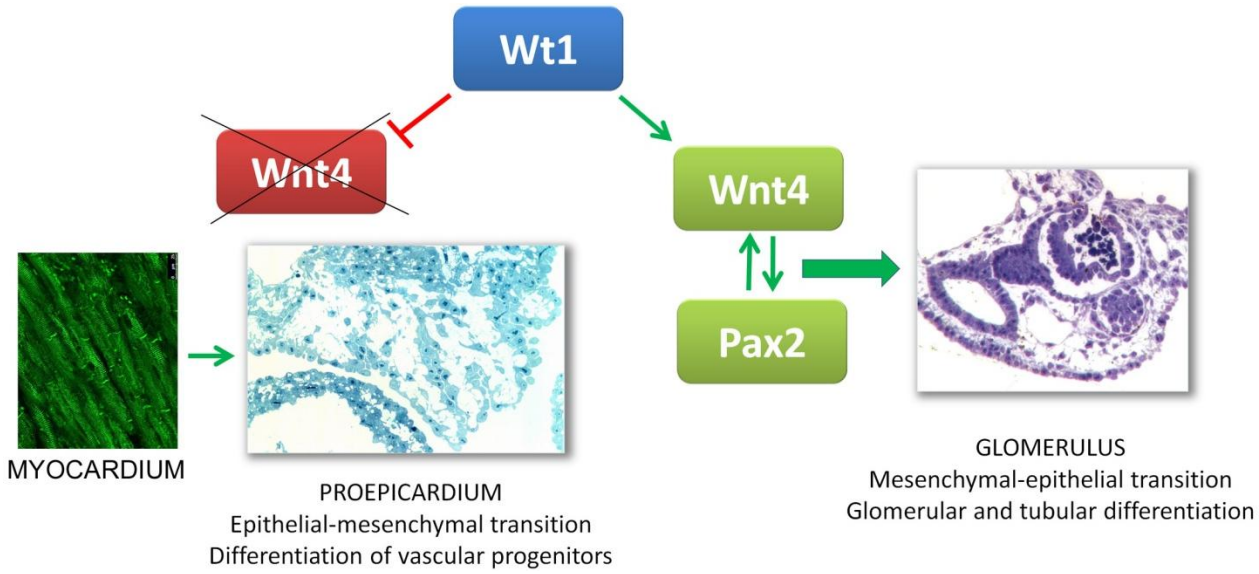
Figure 4. Upregulation of epithelial markers in the proepicardium and epicardium under altered developmental conditions. **(A-C)** Laminin expression appears in the chick proepicardium (PE) after a partial blockade of the proepicardial attachment to the heart by placing a fragment of shell membrane between the proepicardium and the ventricle. In these experimental conditions, the proepicardium is still visible at the stage HH29, when the embryos were fixed. Laminin immunoreactivity appears in inner cells (arrow in A) and it is more prominent in the proepicardial villi located most far away from the heart (arrowhead in B). A glomerulus (GL) of the same embryo, where the strong laminin expression is evident, is shown in C. Note the lack of laminin immunoreactivity in the epicardium (EP) by this stage. **(D-F)** E-cadherin expression is upregulated in the epicardium (arrows) and epicardial-derived cells (arrowhead) in E13.5 Wt1-null mouse embryos (E). The wildtype littermate (D) lacks E-cadherin immunoreactivity in the epicardium by this stage, as shown by the arrows. The negative control performed on the Wt1-null embryo (F) shows that the myocardial staining is non specific.



In summary, a set of genes has probably been evolutionarily conserved in both, the proepicardium and the nephrogenic tissue. Besides these conserved genes, others were lost in the proepicardium, mainly those related with nephric lineage specification like Pax2/8, or with podocyte differentiation (e.g., podocin, unpublished observations). A third set of genes is expressed in the proepicardium and not in the nephric tissue, these are basically genes related with cardiac development. For example, Gata4 is required for proepicardial formation [54], but this gene is not expressed in the fetal

kidney [55]. Knock out of the cardiogenic gene Nkx2.5 results in abnormal proepicardial development and decreased expression of Wt1 [56]. Intermediate mesoderm does not express this gene. However, we think that, although a convergence in gene expression between the proepicardium and the excretory glomeruli cannot be discarded, the coincidences are enough to grant a base for the hypothesis of a common evolutionary origin of these structures.

Figure 5. Cartoon depicting a hypothetical model about how the glomerular differentation of the proepicardium might be inhibited. The lack of Wnt4 expression in the proepicardium together with signals coming from the myocardium promotes epithelial-mesenchymal transition and differentiation of vascular progenitors. On the other side, Wt1 activates Wnt4 expression in the kidneys, and this factor combined with the expression of Pax2 (which is not expressed in the proepicardium) promotes mesenchymal to epithelial transition and glomerular and tubular differentiation. The antagonistic effect of Wt1 on Wnt4 in kidney and proepicardium was demonstrated by Essafi *et al.* [48]. The interaction between Wnt4 and Pax2 is reviewed in Dressler *et al.* [51].



5. The Proepicardium and the Heart-Kidney Complex

We think that the close anatomical relationship between the primordium of the pronephric external glomerulus of the lamprey with the heart, evolutionarily maintained in the proepicardium-myocardium connection, can be revealing a deeper and more ancient relationship between the excretory system and the heart. In fact, hagfishes (myxines), which are phylogenetically much older than lampreys, keep the association of the pronephros and the pericardium in adults, and its glomerulus releases its filtrate into the pericardial cavity [57,58]. This is reminiscent of a structure observed in hemichordates (Figure 3) and known as the heart-kidney complex. This complex is constituted by a pulsatile vessel located in the most anterior part of the body (the proboscis), and a excretory glomerulus formed by vascular spaces lined by a layer of podocytes. The pressure increase provided by vascular contraction of this primitive heart allows for filtration of the blood and release of the filtrate into the proboscis coelomic cavity. Although the hemichordate "heart" is located dorsally, differently to the vertebrate heart, recent

studies on gene expression in the hemichordate *Saccoglossus kowalevskii* have shown that the dorsoventral axis of this species is reversed respect to that of vertebrates [59]. It is important to remark that the expression of a Nkx2.5 ortholog in this hemichordate is localized immediately adjacent to, but caudal to the area where the heart-kidney complex develops [59]. It is tempting to speculate that this observation can be related to the primary and secondary heart fields of vertebrates. The secondary heart field (which is Nkx2.5 negative in vertebrates) contributes to the arterial and venous poles of the heart, and its caudal part is closely related with the expression of nephrogenic markers and it is indeed the place where the proepicardium develops.

There is no structure that can be compared to the heart-kidney complex in non-vertebrate chordates, *i.e.*, urochordates and cephalochordates. However, it is important to remark that a defined excretory system is lacking in urochordates, while the cardiac domain is totally decentralized in cephalochordates [60]. The relationship between excretory and hematopoietic domains in amphioxus is described in the next section.

6. Proepicardium, Kidney and Hematopoiesis

The relationship that we are proposing between the proepicardium and the primitive pronephros may both, to explain and be supported by the striking link between epicardium, kidney and hematopoiesis. Blood-islands have been described in the epicardium of mammalian embryos, including humans [17,61]. Hematopoietic progenitors, basically erythropoietic, have been derived *in vitro* from proepicardial-derived cells [62–64]. Erythropoietin and its receptor are required for correct epicardial development. All these observations can be related with the role played by the pronephros as a main primary hematopoietic organ in fish and amphibians [65,66]. In myxines, the heart-associated pronephros is an adult hematopoietic organ [57].

The association between excretory and hematopoietic domains can be ancestral to vertebrates. We have recently described in amphioxus (*Branchiostoma*) a putative hematopoietic domain characterized by the expression of the hematopoietic markers *Scl* and *Pdvegfr*. This area is closed to the dorsal aorta, partially linked to excretory tissues (characterized by the expression of Pax2/5/8 ortholog, and its development is regulated by retinoic acid [60].

7. Concluding Remarks

We think that our proposal of an evolutionary relationship between pro/epicardium and the excretory system, namely the glomerular compartment of this system, allows for a better understanding of many peculiarities of the epicardial development. Furthermore, this proposal probably is revealing a phylogenetically deeper relationship between the heart and the excretory system, a relationship that still exists in the most primitive vertebrates and it is witnessed by the peculiar way in which the epicardium develops. The relationship between heart and excretory system is not restricted to hemichordates and their heart-kidney complex. Association between a hemal pump and excretory organs can be seen in many invertebrate phyla, including arthropods and molluscs (see phylogenetic position in Figure 3). In the snails, a renopericardial canal drains the excess of pericardial fluid into the kidneys, and in some cases, the heart itself is a source of filtrate for excretion, being the atrial

epicardium composed of podocytes [67–69]. In the cephalopods, the epicardium is continuous with the renal appendages, which constitute the main excretory system [6].

Even in *Drosophila*, which lacks of a blood filtration system, pericardial nephrocytes are arranged along the heart, and derive from the cardiac mesoderm [70]. Incidentally, this mesoderm giving rise to cardiac cells and nephrocytes is also the progenitor of the lymph gland, the source of hemocytes [71]. Thus, the ontogenetic relationship between hemal pumping, excretion and hematopoiesis that we have described above also appears in animal taxa, which are phylogenetically located far away from vertebrates.

The scenario that we are proposing also clarifies the nature of the proepicardium as the primordium of an organ whose differentiation pathway become blocked and their cells are transferred to the heart, where they contribute to the cardiac vascular and connective tissue. For this reason we think that the term "proepicardial organ" is unjustified, since the potential of the proepicardium as a functional organ is never displayed. The only exception are the agnathans, where we cannot talk about a "proepicardium" in strict sense, since the tissue from which the epicardial cells migrate is just a developing glomerulus.

On the other hand, we think that the anatomical relationship between liver and proepicardium is purely contingent, although it is possible that signals inducing expression of proepicardial markers can arise from the liver or, most probably, from the septum transversum mesenchyme where the liver develops. Although evidence of an induction of the proepicardium by the liver has been shown [72], more recently it has been demonstrated that the liver is not required for proepicardial induction in zebrafish [73].

Coronary vessels would differentiate, at least partially, from cells that ancestrally were progenitors of glomerular vessels. It is uncertain if some peculiarities of the coronary vessels could be accounted for this particular origin. For example, Wt1 is upregulated by hypoxia in coronary vessels and kidneys, but not in brain or spleen [74].

Finally, it seems clear that our proposal of an active blocking mechanism repressing the glomerular potential of the proepicardium opens interesting opportunities to test the hypothesis by identifying these mechanisms and circumventing them. In this way it might be conceivable to perform evolutionary rescue experiments transforming the proepicardium into a fully developed external glomerulus, using specific inducing signals and controls to demonstrate that this transformation is due to a conserved potential and not to the pluripotentiality of the proepicardial tissue. These experiments, which we are currently performing in our laboratory, would be a "gold standard" to demonstrate the evolutionary origin of the proepicardium.

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Conflict of Interest

"The authors declare no conflict of interest".

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