Coelomic epithelium-derived cells in visceral morphogenesis

Authors: Laura Ariza[1,2], Rita Carmona[1,2], Ana Cañete[1,2], Elena Cano[3], and Ramón Muñoz-Chápuli[1,2,*]

Addresses:

[1]University of Málaga, Faculty of Science, Department of Animal Biology, 29071 Málaga (Spain)

[2]Andalusian Center for Nanomedicine and Biotechnology (BIONAND), Severo Ochoa nº25, 29590 Campanillas (Spain).

[3]Group of Cardiovascular Genetics, Department of Vertebrate Genomics and Cardiovascular Genetics, Experimental and Clinical Research Center. Charité-Universitätsmedizin Berlin and Max Delbrück Center (MDC) for Molecular Medicine. Lindenberger Weg 80, Berlin 13125, Germany.

***Corresponding author:** Ramón Muñoz-Chápuli, Department of Animal Biology, Faculty of Sciences, University of Málaga, 29071 Málaga (Spain).

Phone: +34-952131853

Fax: +34-952131668

Email: chapuli@uma.es

Running title: Coelomic epithelium in morphogenesis

Grant sponsors. This study was funded by grants BFU2011-025304 and BFU2015 -52299-P (Spanish Ministry of Economy and Innovation), P11-CTS-07564 (Junta de Andalucía), and grants from the Instituto de Salud Carlos III co-founded by Fondo Europeo de Desarrollo Regional - FEDER (contract number:RD06/0019/0022).

Abstract

Coelomic cavities of vertebrates are lined by a mesothelium which develops from the lateral plate mesoderm. During development, the coelomic epithelium is a highly active cell layer, which locally is able to supply mesenchymal cells that contribute to the mesodermal elements of many organs and provide signals which are necessary for their development. The relevance of this process of mesenchymal cell supply to the developing organs is becoming clearer since genetic lineage tracing techniques have been developed in recent years. Body wall, heart, liver, lungs, gonads and gastrointestinal tract are populated by cells derived from the coelomic epithelium which contribute to their connective and vascular tissues, and sometimes to specialized cell types such as the stellate cells of the liver, the Cajal interstitial cells of the gut or the Sertoli cells of the testicle. In this review we collect information about the contribution of coelomic epithelium derived cells to visceral development, their developmental fates and signaling functions. The common features displayed by all these processes suggest that the epithelial-mesenchymal transition of the embryonic coelomic epithelium is an underestimated but key event of vertebrate development, and probably it is shared by all the coelomate metazoans.

KEYWORDS

Coelomic epithelium, mesothelium, epithelial-mesenchymal transitions, epicardium, Wilms' tumor suppressor gene.

Introduction

Vertebrates are coelomate organisms with a coelomic cavity which becomes compartmentalized by septa throughout the evolution. The number of compartments ranges from two in fish (pericardial and peritoneal cavity) to four in mammals (two pleural cavities for the lungs in addition to the former).

All these cavities are lined by a coelomic epithelium which derives from the lateral plate mesoderm. This derivation has been well established, but there is a lack of information in the recent literature about how the coelomic epithelium differentiates. This is probably due to the assumption that the coelomic epithelium is just the cellular lining of the coelomic cavity, and thus the appearance of the coelomic cavities automatically involves the formation of a coelomic epithelium. This is not true, since the mesodermal layers within which the definitive coelomic cavities appear (somatopleura and splanchnopleura) are not "epithelia" strictly speaking, i.e. polarized cells with lateral adhesions and resting on a basal lamina (Figure 1A). The primitive somatopleura and splanchnopleura are very active cell layers giving rise to an abundant population of mesodermal cells which forms the body wall and the mesodermal compartment of many viscera (Figure 1B,C). The splanchopleura also gives rise to the primitive cardiac tube (Figure 1B). Thus, the coelomic epithelium, *sensu stricto*, appears when the adluminal layer of somatopleural and splanchnopleural cells acquires epithelial features, basoapical polarization and basal lamina. The somatopleura gives rise to the coelomic epithelium of the body wall while the splanchnopleura is the origin of the coelomic epithelium lining the organs contained in the coelomic cavity. What is relevant in the context of this review is that this embryonic coelomic epithelium locally becomes activated giving rise to further populations of mesodermal cells as described below (Figure 1D,E)

In adults, this coelomic epithelium, called mesothelium, plays a number of roles. Its primary function is to avoid adherences between tissues and to facilitate lubrication and visceral movements. Furthermore, the adult mesothelium also controls the transport of fluids, cooperates with cells of the immune system and synthesizes cytokines and growth factors (Mutsaers and Wilkosz, 2007). For the sake of clarity, in this review we will call ‘mesothelium’ the fully differentiated, epithelial cell layer of adults and late embryos, and "coelomic epithelium" the embryonic cell layer lining the coelomic cavities which does not show full epithelial differentiation and is able to locally transform into mesenchymal cells.

Despite some works (e.g. Gruenwald, 1942), only recently it has been realized that the embryonic coelomic epithelium has specific functions that in many cases are essential for visceral morphogenesis. This tissue is very active during development, and it can locally transform into mesenchymal cells, here called coelomic epithelium-derived cells (CEDC). CEDC invade the developing viscera, thus contributing to their stroma, differentiating in a number of

cell types and interacting molecularly with the resident tissues. When these functions are experimentally disturbed, severe consequences for the development follow, leading in many cases to embryonic lethality. For example, the embryonic epicardium, which is the coelomic epithelium of the heart, gives rise to a population of epicardial-derived cells which are essential for the development of the coronary vessels and also for the proper development of the ventricular myocardium (Carmona *et al.* 2010). The embryonic gut receive a contribution of cells delaminating from the coelomic epithelium which show the same developmental potential of the periintestinal splanchnopleura (Wilm *et al.* 2005; Carmona *et al.* 2013), and a similar process has been described in the lungs (Que *et al.* 2008; Cano *et al.* 2013a). Stellate cells of the liver and pancreas derive from cells that migrate from the embryonic coelomic epithelium (IJpenberg *et al.* 2007; Asahina *et al.* 2011). Sertoli and Leydig cells from the testicle and granulosa cells from the ovary are also derived from the coelomic epithelium of the genital ridges (Hatano *et al.* 1996; Karl and Capel, 1998; Kusaka *et al.* 2010; Mork *et al.* 2012).

The epithelial-mesenchymal transition (EMT) of the coelomic epithelium seems to be essential for visceral morphogenesis and even for apparently unrelated processes such as limb bud morphogenesis (Gros and Tabin, 2014). Our review emphasizes the developmental significance of the processes of EMT in the coelomic epithelium. Specifically, here, we describe and discuss the most relevant information about the mechanisms leading to the EMT of the coelomic epithelium in different organs, the differentiation potential of the CEDC, the molecular interactions of CEDC with the resident tissues and their global contribution to vertebrate morphogenesis. We think that the generation of CEDC, which has been independently described in different organs, shows common features and mechanisms, and should be regarded under an unifying perspective as a main morphogenetic process in vertebrates and, probably, in all the coelomate metazoans.

Lineage tracing of coelomic-epithelium derived cells

Delamination of cells from the coelomic epithelium was first inferred from morphological features (basal cytoplasmic processes, fragmentation or lack of basal lamina, loss of lateral adhesion) (Figure 2A). The classical work by Gruenwald (1942) provided evidence for the dynamic role played by the embryonic coelomic epithelium of gonads based only on morphological traits. The presence of condensed cytokeratin remains in mesenchymal cells has also been used as a transient marker of a coelomic epithelium origin, since EMT involves loss of the epithelial cytokeratin in the cytoskeleton of the CEDC (Pérez-Pomares *et al.* 1997), but this loss is progressive and the cytokeratin concentrates for some time in a perinuclear dot (Figure 2B). However, in order to establish the developmental fate of the cells derived from the coelomic epithelium it is necessary to durably label these cells in some way. Direct labeling with vital dyes has been proven to be the best procedure available at the time. Lipophilic dyes such as DiI or DiO, as well as more soluble fluorescein derivatives (e.g. CCFSE, carboxy-2',7'-

dichlorofluorescein diacetate, succinimidyl ester, a fixable, cell-permeant tracer) have been used to study EMT in chick embryos (Griffith and Hay, 1992) (Figure 2C,D). DiI staining allowed to establish the coelomic origin of Sertoli cells (Karl and Capel, 1998) while vital staining with Mitotracker has been used to study the coelomic origin of granulosa cells (Mork *et al.* 2012).

In mouse embryos, genetic lineage tracing has been possible in recent years through the Cre-Lox technology (Figure 2F-G). Crossing mice with coelomic-epithelium specific drivers expressing Cre-recombinase with reporter lines such as Rosa26R-YFP or Rosa26R-LacZ allows for constitutive expression of reporters in cells derived from the coelomic epithelium (Mao *et al*., 1999; Soriano, 1999). If the drivers are inducible, for example with tamoxifen administration, it is possible to establish the developmental stage in which the coelomic epithelium becomes labeled (Zhou *et al.*, 2008).

Different drivers have been used for lineage tracing of the embryonic coelomic epithelium. The most widely used have been Cre drivers activated by the *Wt1* (Wilms' tumor suppressor gene) promoter. *Wt1* is strongly expressed by the coelomic epithelial cells in a highly dynamic fashion, allowing for tracing of the CEDC in the areas where *Wt1* is expressed. However, the results obtained with this system must be carefully analyzed since *Wt1* is also expressed in some mesenchymal cells (for example in the intermediate mesoderm) and not all the coelomic epithelium express *Wt1* at a given stage. However, for many organs (heart, lungs, liver, gut), Wt1-Cre is a good option for genetic lineage tracing of the CEDC (Wilm *et al.* 2005; Que *et al.* 2008; del Monte *et al.* 2011; Wessels *et al.* 2012; Carmona *et al.* 2013; Cano *et al.* 2013a).

Mesothelin-Cre has recently been used for the tracing of the CEDC in the trunk, showing a wide contribution of these cells to the fibroblasts and smooth muscle of the trunk, as described below (Rinkevich *et al.* 2012).

Our laboratory has recently used a Cre driver based on the G2-enhancer of the *Gata4* gene (Rojas *et al.* 2005; Delgado *et al.* 2014). This enhancer drives expression of G*ata4* in lateral plate mesoderm and it is highly active in specific areas of the coelomic epithelium, for example in the heart, liver, adrenals and gonads (Figure 2A, 3A,B,D and 4A).

Comparison of the results obtained with all these drivers and other more or less mesothelial-specific (e.g. podocalyxin, cytokeratins) will probably allow for a safe identification of the developmental fate of the CDEC in all the organs.

The best known: Epicardial-derived cells in cardiac development

The cardiac mesothelium is called the epicardium. The heart is not covered by a cardiac mesothelium from the beginning of its development, differently to other organs. Instead, the primitive epicardial cells migrate to the heart from an extracardiac primordium

called the proepicardium. The proepicardium, which can be paired or asymmetrical (Schulte *et al.* 2007; Schlueter and Brand 2009) develops on the posterior limit of the heart, and it is composed of epithelial and mesenchymal cells, probably derived from the coelomic epithelium itself. Depending on the species, the proepicardial cells attach directly to the myocardium or the cells are released into the pericardial cavity where they float and later adhere to the cardiac surface. Finally, the epicardial cells flatten and spread all over the heart (Manner *et al.* 2001; Carmona *et al.* 2010). This unique process of mesothelial formation is probably due to evolutionary reasons that will be discussed in the last part of this section.

The embryonic epicardium gives rise to mesenchymal cells that first populate the subepicardial space (between the epicardium and the myocardium) and then migrate into the myocardium. These epicardial-derived cells (EPDC, a special subtype of CEDC) play essential roles in cardiac development as discussed below. The generation of mesenchymal cells from the embryonic epicardium has been described as being controlled by the process of EMT. However, several differences between the EMT process giving rise to EPDC and the 'classical' EMT processes should be remarked. First of all, the early embryonic epicardium is not a typical epithelium. Their intermediate filaments are composed of cytokeratins but also of vimentin, a feature of mesenchymal cells (Pérez-Pomares *et al.* 1997, 1998). In mouse embryos, typical epithelial features are only displayed by the epicardium in embryos older than E15.5 check laminin, while most EPDC originate between E11.5 and E13.5. Thus, the early steps of EMT (disassembly of cell junction complexes and degradation of basal lamina) apparently do not occur in the embryonic epicardium. A possibility to be considered in order to explain these peculiarities is that epicardial EMT can be regarded as a two-phase process. First, in the developing proepicardium, the coelomic mesothelial cells would give rise to mesenchymal cells through a typical EMT. Then, the mesenchymal cells would be transferred to the heart and part of them would invade the myocardium. The flat cells remain on the surface and later reacquire epithelial features in a mesenchymal to epithelial transition. This two-phase process would explain, for example, why Snail expression is dispensable in the epicardium for the EMT (Casanova *et al.* 2013). On the other hand, it has been described that EMT is actively inhibited during the phase of epicardial migration, possibly through integrin-mediated signaling (Dokic and Dettman 2006).

Thus, the peculiarities of the epicardial EMT would be associated with the unique way in which the epithelial lining of the heart originates from a primordium which develops independently of the organ. Despite these peculiarities, a number of factors involved in other processes of EMT are also acting on the embryonic epicardium. For example *Wt1*, which is strongly expressed by proepicardial and epicardial cells (Figure 3A), seems to be required for epicardial EMT, since its conditional deletion in these cells leads to impaired generation of EPDC and coronary artery development (Martínez-Estrada *et al.* 2010). This effect of Wt1 might be mediated by induction of Snail and down regulation of E-cadherin. However, as

stated above, Snail might be dispensable in the epicardium for EMT (Casanova *et al.* 2013) although E-cadherin appears increased in the epicardium when Wt1 is down regulated (Martínez-Estrada *et al.* 2010). Alternatively Wt1 could be controlling epicardial EMT by regulating canonical Wnt and retinoic acid (RA) signaling (von Gise *et al.* 2011).

The myocardium provides signal(s) for migration of EPDC from the epicardium. This is demonstrated by myocardial inactivation of *Fog2*, a GATA cofactor, which results in a lack of EPDC in the ventricle despite normal epicardial covering (Tevosian *et al.* 2000). Migration of EPDC is stimulated by growth factors such as TGFβ1-3 (Sánchez *et al.* 2011; Dokic and Dettman, 2006; Austin *et al.* 2008), PDGF-BB (Lu *et al.* 2001), bFGF (Dettman *et al.* 1998; Morabito *et al.* 2001) or VEGF (Nesbitt *et al.* 2009). TFGβ signaling is a well-known inducer of EMT in other systems, such as the endocardial cushions (reviewed in Kruithof *et al.* 2012). The conditional deletion of the receptors of some of these factors in the epicardium supports their role in EPDC migration, for example the Type-I TGFβR (ALK5) (Sridurongrit *et al.* 2008) or PDGFRα-β (Mellgren *et al.* 2008, Smith *et al.* 2011). PDGFRs are also down-regulated in *Wt1*-deficient epicardium (Guadix *et al.* 2011). Finally, double deletion of FGFR1/FGFR2b receptors in the epicardium does not abolish EMT but the migrating ability of EPDCs is much impaired (Vega-Hernández *et al.* 2011).

RA signaling also plays a role in epicardial EMT, since conditional epicardial deletion of the receptor RXRα impairs EMT and coronary development (Merki *et al.* 2005). Wt1 induces *Raldh2* expression in the heart (Guadix *et al.* 2011) and Wt1 deletion in the epicardium reduces RA signaling (von Gise *et al.* 2011). Downstream of RA, factors such as FGFs or WNTs also participate in epicardial EMT. RA regulates FGF9 and WNT9b expression in epicardium through RXRα, and these factors are involved in epicardial EMT (Merki *et al.* 2005). It is interesting to remark that areas of the embryonic coelomic epithelium where EMT is active usually show high expression of the RA synthesizing enzyme RALDH2 (Figure 3F).

An important question that remains to be addressed is the mechanism that stabilizes the epicardium and prevents epicardial EMT in late embryos and adults. The interaction between myocardial VCAM-1 and epicardial α4β1 integrin seems to be involved in this stabilization (Dettman *et al.* 2003), probably by inhibiting cytoskeletal changes (Dokic and Dettman, 2006). These changes can be related with the control of RhoA/Rho kinase activity, which has been recently involved in the maintenance of the epicardial EMT (Artamonov *et al.* 2015).

EPDC, as stated above, play essential roles in cardiac development. Evidence has been provided about differentiation of EPDC into fibroblasts, smooth muscle, endothelium and myocardial cells, although the latter contribution is more controversial (Rudat and Kispert, 2012). It has been well established that a majority of smooth muscle cells of the coronary vessels are derived from EPDC (Dettman *et al.* 1998; Vrancken Peeters *et al.* 1999).

Cardiac fibroblasts produce multiple components of the extracellular matrix of the heart, including collagens and fibronectin, and constitute the adventitial layer of the coronary vessels. Lineage tracing studies have demonstrated the contribution of EPDC to the cardiac fibroblast population, although alternative origins of fibroblasts (e.g. endocardium and bone marrow) have also been described (reviewed in Krenning *et al.* 2010). EPDC also give rise to a portion of the fibroblasts of the endocardial cushions, together with the cells derived from the endocardium (Wessels *et al.* 2012). Finally, the proepicardial origin of a cardiac population of pluripotent colony-forming units - fibroblast (CFU-Fs), analogous to the mesenchymal stem cells, has also been proposed (Chong *et al.* 2011).

The differentiation of EPDC into coronary endothelial cells has been largely controversial. Although quail-chick chimaera studies demonstrated this epicardial contribution, some lineage tracing studies first discarded this possibility in mice. More recent studies have shown that the coronary endothelium is a mosaic composed in its majority of endocardial cells, but with a significant contribution of EPDC (Katz *et al.* 2012; Wu *et al.* 2012) (Figure 3C). We have recently estimated that EPDC contribute about a quarter of the total number of coronary endothelial cells of the ventricle by the end of the gestation (Cano *et al.* submitted). These coronary endothelial cells from epicardial origin probably play a key role in the formation of the coronary arteries and in arterio-venous patterning, thus explaining the severe vascular defects observed in models of loss of function of epicardial specific genes.

Finally, the contribution of EPDC to myocardium was ruled out at least in avian embryos by quail-chick chimaera experiments (Manner, 1999) although the chick proepicardium keeps a myocardiogenic potential *in vitro* (Kruithof *et al.* 2006). Evidence of generation of cardiomyocyes from EPDC in mouse embryos has been provided by different lineage tracing studies (Cai *et al.* 2008; Zhou *et al.* 2008) although the issue remains controversial (Rudat & Kispert, 2012; Zhou & Pu, 2012).

EPDC are not only essential for cardiac development due to their potential of differentiation into a number of cardiac cell lineages but also by their signaling properties. EPDC and the epicardium are a source of factors which induce myocardial proliferation and maturation, and the lack of these factors accounts for part of the severe phenotype resulting in inactivation of genes involved in epicardial development. This phenotype is frequently characterized by thin ventricular myocardium, leading to cardiac failure and embryonic death.

A number of paracrine factors have been identified as produced by epicardium and EPDC. IGF2 is a potent mitogen secreted by the epicardium during midgestation which activates the ERK proliferation pathway in the myocardium (Li *et al.* 2011). Erythropoietin (Epo) and its receptor EpoR are expressed in the epicardium, and their deficiency leads to thin myocardium (Stuckmann *et al.* 2003). Epo is regulated in the epicardium by Wt1 and probably induces secretion of IGF2 (Brade *et al.* 2011). RA is produced through RALDH2 in the

epicardium and EPDC as stated above. Although RA seems not to promote myocardial proliferation directly, the RA signaling pathway is essential for the production of some of the epicardial factors inducing myocardial proliferation, basically Epo and members of the IGF and FGF families of growth factors (Kubalak & Sucov, 1999; Brade *et al.* 2011).

The epicardium, which represents the best known instance of coelomic epithelium contribution to a developing organ, shows a number of peculiarities that distinguish the embryonic epicardium from any other embryonic coelomic epithelium, as we will see in the next sections. We have proposed that most of these differences can be accounted by the special evolutionary origin of the proepicardium (Pombal *et al.* 2008; Cano *et al.* 2013b, Cano *et al.* 2015). We hypothesize that the proepicardium derives from an ancestral pronephric external glomerulus which lost its excretory function but maintains the ability to provide the heart with progenitor cells for vessels and connective tissue. In fact, epicardial cells derive, in the embryos of phylogenetically primitive lampreys, from the primordium of a pronephric external glomerulus which later becomes fully functional (Pombal *et al.* 2008). It is important to consider that, differently to any other viscera, the primitive cardiac tube derives from the splanchnopleural sheet, the same tissue which originates the coelomic epithelium and the CEDC in other organs, as we are reviewing in this paper. Thus, the myocardium can only be supplied by CEDC through a specific mechanism and through an extracardiac tissue. The proximity of the filtering system based on external glomeruli and the heart in primitive vertebrates provided such specific mechanism for mesothelial lining. We can explain in this way the high number of common genes required for development of epicardium and kidneys (reviewed in Cano *et al.* 2013b). As a support for this hypothesis we have partially rescued the primitive glomerular fate of the proepicardium by exposing chick cultured proepicardium to high concentrations of RA, a treatment that leads to an upregulation of intermediate mesoderm and glomerular genes (Cano *et al.* 2015).

Coelomic epithelium-derived cells and lung development

The primary lung buds which appear in the mouse at E9.5 and in humans at day 28 (Morrisey & Hogan, 2010), consist of an inner epithelium of endodermal origin surrounded by a packed mesenchyme and a covering coelomic epithelial layer, both being mesodermal derivatives (Zorn & Wells, 2009). Diffusible signals from the surrounding mesenchyme and the coelomic epithelium, belonging to the FGF, TGFβ, BMP, Hedgehog, Wnt, and EGF families, are essential to direct the pulmonary morphogenetic program (Wells & Melton, 2000; Weaver *et al.* 2003). Coelomic epithelial cells secrete a number of signaling molecules such as FGF9, TGFβ3 and RA which regulate the growth and patterning of distal mesenchyme (Dickman *et al.* 1997; Malpel *et al.* 2000; Bragg *et al.* 2001; Colvin *et al.* 2001; White *et al.* 2006; Rawlins, 2011).

Que *et al.* (2008) pointed the coelomic epithelial layer as a source of mesenchymal cells. Using a Wt1-Cre transgenic mouse model (Tg(WT1-cre)AG11Dbdr) and direct labeling (CCFSE), they described that a portion of the early pleural coelomic epithelium undergoes EMT and gives rise to CEDCs which migrate into the lung bud and differentiate into vascular smooth muscle cells. In this study, the authors reported that an average of 30% of vascular smooth muscle cells are derived from Wt1-expressing coelomic epithelial cells. Likewise, the authors suggested a possible mesothelial origin for interstitial fibroblasts, alveolar myofibroblasts and even a few endothelial cells, although no conclusive experimental evidence was provided. The contribution of CEDC to the vascular smooth muscle cell layer was further confirmed by other groups using different Wt1-Cre (mWt1/IRES/GFP-Cre in Cano *et al.* 2013a; Wt1tm2(cre/ERT2)Wtp in Dixit *et al.* 2013) and mesothelin-Cre transgenic mouse lines (MSLN–CreER^{T2}–IRES–lacZ) (Rinkevich *et al.* 2012) (Figure 2E-G). In contrast, Greif *et al.* (2012) studied specifically the origin of the smooth muscle wall of the developing pulmonary artery and reported no contribution of Wt1-lineage coelomic epithelial cells to the vascular wall of this specific pulmonary vessel, stating that the unique origin of the media layer of the lung vasculature is the PDGF-B+ mesenchyme (Greif *et al.* 2012). Nevertheless, various authors have reported that the recombination efficiency of the tamoxifen-inducible Wt1-Cre transgenic line (Wt1tm2(cre/ERT2)Wtp) used by Greif *et al.* (2012) and Dixit *et al.* (2013) is highly variable depending on the strain background and tamoxifen administration route (Rudat & Kispert 2012; Zhou & Pu 2012).

Using a mWt1/IRES/GFP-Cre transgenic line (del Monte *et al.* 2011, Wessels *et al.* 2012, Carmona *et al.* 2013), Cano *et al.* (2013a) confirmed with live imaging of embryonic lungs the migration of CEDC into the lung bud. In this study, the potential of Wt1-lineage CEDC to differentiate into diverse cell types was further analyzed. According to these results, the potential of the pleural coelomic epithelium is much wider than previously described, Wt1-lineage CEDC do not only contribute to the vascular smooth muscle layer, but also significantly to the endothelial and perivascular layers of the lung vasculature. Additionally, Cano *et al.* (2013a) the contribution of Wt1-lineage CEDCs to the visceral smooth muscle layer covering the bronchial walls and to the fibromuscular and cartilaginous elements of the bronchi. As far as we know, this is the first time that CEDCs were described to give rise to chondrocytes. These authors proposed that the pulmonary mesenchyme has a double origin: first, from the preexisting splanchnopleural mesoderm that surrounds the endodermal sprouts, and second, from the coelomic epithelium lining the lung buds, which is characterized by Wt1 expression.

The contribution of Wt1-lineage CEDC to bronchial smooth muscle layers and to interstitial fibroblasts has been supported by various studies (Dixit *et al.* 2013; Cano *et al.* 2013a). However, different results have been published regarding the contribution of CEDCs to fibroblasts. The fibroblast population shows an enormous phenotypic variability, given the heterogeneity of the cells that can be considered fibroblasts and the dynamic gene expression

profile that this population exhibits. Indeed, specifically in the lungs, fibroblasts have been shown to be heterogeneous in cell surface marker expression as well as in the levels of collagen production (Fries *et al.* 1994). Dixit *et al.* (2013) reported differentiation of Wt1-lineage CEDCs to desmin-expressing fibroblasts during embryonic and postnatal lung development. On the other hand, Cano *et al.* (2013a) observed that the CEDC contribution to 5B5-expressing or FSP1-expressing fibroblast was very rare in embryonic and neonatal stages. Interestingly, the FSP1+ fibroblast subpopulation was shown to be derived from bone-marrow circulating progenitors (given the coexpression of FSP1 and the panleukocytic marker CD45). These authors showed for the first time that the recruitment of circulating progenitors to give rise to FSP1 pulmonary fibroblast does not only occur during fibrosis process in the adult, but also during fetal life. Additionally, Cano *et al.* (2013a) showed that Wt1-lineage CEDC differentiate into a non-endothelial, non-hematopoietic, fibroblast-like CD34+ cells at late gestational stages. They could be the same interstitial fibroblast-like cells described in the gastrointestinal tract (see next section). The presence of this population in the lung opens new questions about its possible function.

Pulmonary endothelial cells progenitors have been identified in developing mouse lung at E10.5, as a layer of mesenchymal cells surrounding the epithelial tube. This layer coalesces into a primary capillary network which develops into a more definite vascular network (deMello *et al.* 1997; Schachtner *et al.* 2000). Cano *et al.* (2013a) demonstrated that two different components, the splanchnopleura and the coelomic epithelium, contribute to these progenitors. Likewise, Wt1-lineage CEDCs are found in part of the smooth muscle and perivascular layers of the pulmonary blood vessels. The number of endothelial cells derived from the Wt1-expressing coelomic epithelium increases significantly until midgestation, reaching 25% of total endothelial cells, and declines by the end of gestation, accounting for about 3% of all the pulmonary endothelial cells in neonatal stage. According to these authors, this decrease in the number of coelomic epithelium-derived endothelial cells is due to recruitment of angioblasts from other sources, most likely from the circulation or from the lung mesenchyme itself.

The systemic *Wt1* knockout embryos (described in Kreidberg *et al.* 1993) show defects in the lungs, which exhibit an irregular rounded-shape and abnormally fused lobes (Cano *et al.* 2013a). Although it cannot be discarded that these abnormalities are secondary defects due to a malformed pleural cavity, the EMT process is not taking place normally in the lungs, as demonstrated by the reduction of vimentin immunoreactive cells in the lung mesenchyme and the presence of an intact basal lamina under the pulmonary coelomic epithelium of the *Wt1* knockout embryos.

Coelomic epithelium-derived cells in the development of the gastrointestinal tract

The primitive endoderm and the surrounding splanchnic mesoderm generate the entire digestive tract and its derivatives, liver, bile ducts and pancreas (Wells and Melton, 1999). The digestive tract is later colonized by the neural crest cells which differentiate into neurons of the enteric plexus (Young *et al.* 2000; Young and Newgreen, 2001; McLin *et al.* 2009). The basic structure of the gastrointestinal tract (GIT) is conserved among vertebrates and consists of an epithelial mucosa, submucosa, muscularis externa and a coelomic epithelium (also called serosa in adults) (Netter, 1997; Roberts *et al.* 1996).

In recent years, several studies have revealed the existence of a process of EMT in the embryonic coelomic epithelium of the GIT, giving rise to a CEDC population which mixes with the preexisting mesodermal cells that surround the endoderm. Wilm *et al.* (2005) were the first to describe the existence of a process of EMT in the mesothelium lining the intestine. Using a Wt1-Cre transgenic mouse line (Tg(WT1-cre)AG11Dbdr) and direct labeling (CCFSE), these authors demonstrated that a subpopulation of CEDC differentiates into vascular smooth muscle cells of all major blood vessels in the GIT. More recently, Carmona *et al.* (2013) demonstrated, with a model based on mWt1/IRES/GFP-Cre mice (also used in other studies as stated above), that cells from the Wt1 lineage play multiple and dynamic roles in intestinal development. In contrast to previously published data, these authors showed evidence that Wt1-lineage cells, derived from the coelomic epithelium, are able to differentiate into endothelium, vascular and visceral smooth muscle and interstitial cells of Cajal (ICC).

ICC constitute a cellular network widely distributed within the submucosal, intramuscular and intermuscular layers of the GIT (Ward 2000; Takaki 2003). These cells are defined by the expression of c-Kit (the receptor for the stem cell factor SCF) (Maeda *et al.* 1992; Torihashi *et al.* 1995) and anoctamin-1 (Gómez-Pinilla *et al.* 2009; Sanders *et al.* 2012). The origin of the ICC has been thoroughly discussed and it was proposed that they could derive from the neural crest (Le Douarin and Teillet, 1973), the gut mesenchyme (Lecoin *et al.* 1996; Young *et al.* 1996) or the smooth muscle cells of the GIT (Kluppel *et al.* 1998). Carmona *et al.* (2013) showed, by immunohistochemistry and RT-PCR, that at least part of the ICC population is derived from CEDC (Figure 4C). Other work described a cell population in the submucosal layer, characterized by their expression of CD34 and called interstitial Cajal-like cells (Vanderwinden *et al.* 2000; Pieri *et al.* 2008). Carmona *et al.* (2013) showed that part of this population is also derived from the Wt1 expressing cell lineage.

Coelomic epithelium-derived cells and liver development

As mentioned before, the GIT is regionalized through reciprocal interactions with the surrounding mesenchyme that determines the site of organ formation (Zorn and Wells, 2009;

Wells & Melton, 1999). At E9.5 in mouse, the liver is formed as a diverticulum of the foregut endoderm. The ventral region of the foregut endoderm invades the septum transversum mesenchyme (STM) and gives rise to hepatoblasts that are capable of differentiating into both hepatocytes and biliary epithelial cells (Zaret 2002; Si-Tayeb *et al.* 2010). The developing liver is vascularized by a vasculogenic process that leads to the differentiation of the sinusoids (DeRuiter *et al.* 1993; Gouysse *et al.* 2002). The main structural elements of the sinusoidal wall are endothelial cells and stellate cells (also known as Ito cells). The stellate cells surround the endothelial cells, and play supporting functions similar to those performed by pericytes or smooth muscle cells in vessels from other tissues (Enzan *et al.* 1997). Further, stellate cells have other specific functions such as storage of retinoids (Blomhoff *et al.* 1990).

The outer layer of the liver is formed by coelomic epithelial cells. Using direct labeling and immunohistochemical techniques, it was shown in chick embryos that the coelomic epithelium gives rise to mesenchymal cells that delaminate from the epithelium and contribute to the sinusoidal endothelium (Pérez-Pomares *et al.* 2004). These results are consistent with the hypothesis proposed more than three decades ago by Nicole Le Douarin (Le Douarin, 1975). The origin of hepatic endothelium is relevant since it has been shown that in *Flk1* deficient mice, which lack endothelial cells around the hepatic endoderm and in the STM, hepatic specification occurs, but liver morphogenesis fails (Matsumoto *et al.* 2001). *In vitro* experiments performed by these authors showed that the endothelial inductive role was specifically on the endodermal outgrowth, not affecting to the fibroblasts or the expression of early liver genes. Since at least a part of these endothelial cells are CEDCs, these results suggest a function of CEDCs in liver morphogenesis.

Cassiman *et al.* (2006) demonstrated that mouse hepatic stellate cells do not derive from the neural crest. IJpenberg *et al.* (2007) studied the phenotype of the systemic Wt1 knockout mice and described liver hypoplasia with defects in hepatic lobe formation. This study showed that Wt1 was required for the control of differentiation of the coelomic epithelium-derived stellate cell progenitor population, as well as for the activation/maintenance of a RA signaling pathway essential for liver morphogenesis.

Using an inducible Wt1-Cre mouse line (Wt1tm2(cre/ERT2)Wtp), Asahina *et al.* (2011) showed that coelomic epithelial cells surrounding the liver generate, through a process of EMT, mesenchymal cells that invade the liver and differentiate into stellate and perivascular mesenchymal cells. These authors failed to find a contribution of Wt1-lineage cells to endothelial cells of the hepatic sinusoids (Asahina *et al.* 2011). However, unpublished results obtained in our laboratory using a constitutive Wt1-Cre mouse line (mWt1/IRES/GFP-Cre, references quoted above), suggest that CEDC contribute to the endothelium of the hepatic sinusoids (Figure 4B). The discrepancy with the results published by Asahina *et al.*(2011) could be due to a different recombination efficiency caused by differences in dosage and/or route of

tamoxifen administration in the inducible Wt1-Cre mouse line. Further studies will be needed to solve this issue.

Besides its role as a source of mesenchymal cells, the coelomic epithelium of the liver has also a signaling function. Early coelomic cells of the liver are characterized by the expression of podocalyxin-like protein-1 (PCPL1) and the secretion of growth factors such as midkine, pleiotrophin and HGF. These factors are involved in the induction of hepatoblast proliferation and their expression is controlled by wt1 (Onitsuka *et al.* 2010).

Coelomic epithelium-derived cells and pancreas development

The pancreas develops by an inductive process between the endodermal lining of the duodenum and the adjacent splanchnic mesoderm. In mice, the first signs of pancreatic morphogenesis occur at E9.0 when two rudiments, dorsal and ventral, become visible in the primitive gut endoderm. At mid-gestation, pancreatic primordia undergo a reorientation and merge to create a single organ (Deutsch *et al.* 2001; Tremblay *et al.* 2005).

Although the pancreas, like other visceral organs, is covered by a coelomic epithelium layer, the existence of an EMT process with formation of CEDC has never been demonstrated, at least during embryonic development. However, deletion of the *Wt1* gene in adult mice causes atrophy in the pancreas due to increased apoptosis (Chau *et al*. 2011). These authors showed expression of *Wt1* in adult pancreatic stellate cells, which are part of the exocrine pancreas and share many characteristics with stellate cells of the liver, such as the expression of common markers such as desmin (Apte *et al.* 1998). It is uncertain if pancreatic stellate cells and/or endothelial cells derive from the embryonic coelomic epithelium as it occurs in the developing liver. Thus, it becomes necessary to perform studies on the contribution of the coelomic epithelium to developing pancreas, the existence of an EMT process in this epithelium and the potential of differentiation of the resulting mesenchyme.

Coelomic epithelium-derived cells and spleen development

The spleen is located on the left side of the abdominal cavity between the fundus of the stomach and the diaphragm, and it has important functions in haematopoiesis and in the generation of primary immune responses (Burn *et al.* 2008).

Spleen development starts at approximately E10.5-E11 as a thickening of the coelomic epithelium, the splanchnic mesodermal plate (SMP). This plate is a thickening of the coelomic epithelium constituted by a layer of organized, elongated epithelial-like cells. It is located on both right and left sides of the foregut. The cells comprised in the SMP are considered splenic precursors and promote morphogenesis (Green, 1967; Brendolan *et al.* 2007).

By E10-10.5 the SMP loses its thickness on the right-hand of the gut. Cellular proliferation within the SMP on the left side appears to drive the growth of this region towards the left. At E10.5, the population of mesenchymal cells underlying SMP on the dorsal left side expands considerably and acquires splenic cell fate (Brendolan *et al.* 2007). A visible spleen primordium is formed at E13.5 and the definitive spleen in position lateral to the stomach appears at E15.5 (Brendolan *et al.* 2005).

Searle (1964) described the effects of gene dominant hemimelia (Dh) in mice that cause abnormalities of the hind limbs, absence of the spleen and a small and defective digestive tract. Mouse embryos heterozygous for *Dh* mutation are asplenic, but they show normal pancreas (Green 1967; Brendolan *et al.* 2007). In *Dh/Dh* mutant embryos the SMP is not apparent and the organized splanchnic mesoderm is replaced by an unorganized mesenchyme (Hecksher-Sorensen *et al.* 2004). *Wt1* and *Pbx1* expression are detected in the mesenchyme and in the coelomic epithelium that probably gives rise, at least in part, to the prospective spleen capsule (Burn *et al.* 2008). *Wt1*-null embryos show almost complete lack of the spleen due to apoptosis within the spleen mesenchyme (Herzer *et al.* 1999). All these results suggest the coelomic SMP plays a crucial role during spleen development. However, as in the case of the pancreas, we think that studies demonstrating the existence of CEDC in the developing spleen and their contribution to the development of this organ are required.

Coelomic epithelium-derived cells and genitourinary development

The coelomic epithelium located on both sides of the dorsal mesenterium plays a critical role in the development of the gonads, adrenal glands and excretory system. The genital ridge (more medial) and the nephric ridges (more lateral) appear in this area of the intermediate mesoderm by E9.5 in mouse embryos.

The expression of genes which are critical for the development of the urogenital system frequently starts in the coelomic epithelium of the ridges and this expression later appears in the underlying mesenchyme suggesting delamination of this mesenchyme from the surface epithelium. Steroidogenic factor-1 (*Sf-1*) is first expressed in a definite area of the coelomic epithelium, and then in the corticoadrenal stroma, Sertoli and Leydig cells of the testicle and granulosa cells of the ovary (Hatano *et al.* 1996). *Osr1*, *Raldh2* and *Wt1* are also first expressed in coelomic epithelium and later in mesenchyme of the genital and nephric ridges (Bohnenpoll *et al.* 2013). An origin of Sertoli cells from the coelomic epithelium of the genital ridges was first proposed by Karl and Capel (1998). Granulosa cells of the ovary are also derived from coelomic epithelium of the bipotential gonad primordium. However, their origin is more complex since there are two waves of granulosa cell progenitors, before and after birth (Mork *et al.* 2012).

The migration of coelomic cells to the mesenchymal compartment of the gonad primordium was described by Kusaka *et al.* (2010), who demonstrated that this migration is dependent on the expression of the paired-box gene *Emx2*. They also demonstrated that the CEDC expressed SF1 and differentiate into Sertoli and Leydig cells. Thus, the coelomic epithelium contributes to a substantial part of the mesenchymal cells which will constitute the stroma of gonads and adrenal cortex. Proliferation of early CEDC in the XY gonad is induced by the male sex determining gene *Sry*. Interestingly, this proliferation during a specific time window is necessary for differentiation of Sertoli cells and initiation of the male pathway (Schmahl and Capel, 2003).

The relationship of the Mullerian duct, which forms the female reproductive tract, with the coelomic epithelium and the Wolffian duct has been controversial. A detailed study in chicken and mouse embryos concluded that all the components of the Mullerian duct were derivatives of the coelomic epithelium and discarded contribution from the Wolffian duct. CEDC would also contribute to the mesenchymal component of the uterovaginal canal which gives rise to the uterine smooth muscle (Guioli *et al.* 2007).

The coelomic contribution to the nephric ridges has been studied less, and is probably not as relevant as the contribution to the adrenogonadal tissue. In fact, we have observed in the G2-Gata4-Cre model above described that activation of *Gata4* by the G2 enhancer occurs in the coelomic epithelium related with gonads and adrenals but not in the coelomic epithelium of the nephric ridge. Consequently cells from the G2-Gata4 lineage are abundant in gonads and adrenals but not in the mesonephros (Figure 4A). Of note, GATA4 is required for the formation of the genital ridge and for proliferation and migration of CDEC into the gonad (Hu *et al.* 2013).

Wolffian ducts form through mesenchymal to epithelial transition from the intermediate mesoderm mesenchyme and apparently CEDC are not involved in this process (Shaw and Renfree, 2014). Instead, coelomic epithelium of the nephric ridges could have a signaling role. In chicken embryos, the formation of mesonephric ducts and the adjacent coelomic cells sheet proceeds in a coordinated manner. When the Wolffian duct is surgically ablated, the coelomic epithelium exhibits aberrant morphology and increased tendency to EMT. Thus, stability of the coelomic epithelium depends on the presence of the Wolffian duct (Yoshino *et al.* 2014).

Contribution of coelomic epithelium-derived cells to other tissues

We have described how CEDC contribute along embryonic development to fibroblasts and smooth muscle (either visceral or vascular) of organs such as lungs, heart or gastrointestinal tract. This seems to be a general process of development, affecting also the body wall. By using a mesothelin-cre driver (MSLN–CreERT2–IRES–lacZ) Rinkevich *et al.* (2012)

have shown that embryonic and adult mesothelium represent a common lineage for fibroblasts and smooth muscle of the trunk, including the aortic smooth muscle. The contribution of CEDC to the dorsal aorta had been described in chick embryos (Pérez-Pomares *et al.* 1999). Thus, the well known contribution of neural crest cells to the aortic wall seems to be restricted to the section of this artery most proximal to the heart. On other other hand, Rinkevich *et al.* (2012) have also reported CEDC in the stromal compartment of the thymus.

From the evidence obtained from the models of genetic lineage tracing we think that a substantial part of the mesenchyme of the pericardium, STM and pleuroperitoneal folds also has a coelomic epithelium origin (Figure 3E). Alteration of the EMT in this area can probably lead to congenital diaphragmatic hernia, a malformation which has been related with Wt1 function (Dingemann *et al.* 2011; Paris *et al*., 2015; unpublished results).

Mesothelial progenitors contribute also to the visceral adipose tissue surrounding many viscera (but not the subcutaneous or the brown adipose tissues). Differentiation of visceral adipocytes from mesothelial cells also occurs in the adult life (Chau *et al.* 2014). This observation is relevant given the abundance of mesenchymal stem cells in the adipose tissue.

Finally, it is important to quote the recent discovery of an unexpected function for CEDC in the limb bud formation. A localized EMT of the coelomic epithelium gives rise to a mesenchymal population which starts development of the limb buds (Gross and Tabin, 2014).

EMT in adult mesothelium

Some pathologic processes, as liver cirrhosis, endometriosis and serosa inflammation, are associated with an increase of the number of cells present in peritoneal effusions, probably due to a release of cells from a reactive mesothelium. In culture, these released cells generally exhibit a normal epithelial morphology. However, after several passages in culture these cells acquire a fibroblastic phenotype, reducing levels of cytokeratin and increasing vimentin expression (Mackay *et al.* 1990). Additionally, several growth factors have been described to induce mesothelial cells to change their phenotype into a fibroblastic one, adopting an increased motility and enhancing the production of extracellular matrix. In human peritoneal mesothelial cells obtained after dialysis, EGF induces reversible phenotype modifications, leading to an increase of β1-integrin expression, thereby facilitating adhesion and migration through collagen (Leavesley *et al.* 1999). Likewise, EGF, together with PDGFα and Interleukin 10β, produces an increase of collagen production by mesothelial cells (Owens & Milligan, 1994; Yang *et al.* 2003). TGFβ also induces EMT *in vitro*, producing an up-regulation of smooth muscle actin and type I collagen expression (Yang *et al.* 2003). It has been suggested that these different morphologies represent different stages of differentiation of mesothelial cells. Wt1

and certain proteoglycans have also been proposed as an indicator of the progress of differentiation (Gulyás *et al.* 1999).

In adult human biopsies from serosa, Whitaker *et al.* (1992) were the first who suggested that mature mesothelial cells could acquire a fibroblastic phenotype and invade the connective tissue of the subserosal layer *in vivo*. They propose that this process could explain the pattern of intermediate filament expression described by Bolen *et al.* (1987): mesothelial cells express cytokeratins of low and high molecular weight and isolated submesothelial cells express vimentin, whereas reactive submesothelial cells coexpress cytokeratins and vimentin. On the contrary, further evidence pointed that mesothelial cells rarely become mature fibroblasts except during wound repair or tumor progression (Haney, 1993; Yáñez-Mó *et al.* 2003).

Two studies about the pathologic effects of the continuous ambulatory peritoneal dialysis (CAPD) have provided important evidence that supports EMT in adult mesothelium *in vivo*. Under specific stimuli, such as peritoneal injury, the mesothelial cells adopt a fibroblastic phenotype, and they are released into the peritoneal cavity. Mesothelial cells lose their epithelial morphology, reduce cytokeratin and E-cadherin expression and acquire a migratory phenotype with overexpression of α2-integrins (Yáñez-Mó *et al.* 2003; Yang *et al.* 2003). It has been demonstrated that EMT in these adult peritoneal cells is mediated by Snail, which leads to a dramatic down-regulation of E-cadherin and cytokeratin (Yáñez-Mó *et al.* 2003).

Adult mesothelial cells may preserve embryonic pluripotency. The existence of pluripotent mesenchymal cells in the adult mesothelial monolayer, as well as in the submesothelial connective tissue, has been repeatedly considered in the literature. However, new evidence suggests that the mesothelial cells themselves may be multipotential and have the ability to differentiate into various different cell types. This raises the question whether we can consider the mesothelium and CEDCs cells as true stem cells. Mesothelial cells are endowed with a remarkable self-regenerating capability and with a degree of plasticity that allow them to generate certain cell types in the appropriate microenvironment. In embryonic stages, we have shown that its potential of differentiation into different lineages is notable. In adult life, the literature provides some evidence which suggest that this potential and plasticity could persist, confirming, in many cases also in humans, the ability of differentiation into many fates (Fadare *et al.* 2002; Wada *et al.* 2003; Compton *et al.* 2006; Kawaguchi *et al.* 2007; van Tuyn *et al.* 2007; Lansley *et al.* 2011).

CEDC differentiation into smooth muscle cells seems to be remarkably efficient also in adult stages. It has been described that human epicardial cells spontaneously undergo EMT and adopt smooth muscle features after subculturing at low densities (van Tuyn *et al.* 2007). Rat adult epicardial cells have also been shown to differentiate into smooth muscle cells (Wada *et al.* 2003). Peritoneal explants from mouse and chick also show this ability to give rise

smooth muscle cells with some growth factors supplementation (Compton *et al.* 2006; Kawaguchi *et al.* 2007).

Additionally, the ability of adult mesothelial cells to give rise to chondroblast, osteoblast and adipoblast lineages has been addressed in a number of studies. Under osteogenic stimuli, human epicardial cells are able to form calcium deposits *in vitro* (van Tuyn *et al.* 2007). Differentiation into osteoblast and adipocyte-like cells of primary human and rat mesothelial cells has also been described (Lansley *et al.* 2011). Additionally, biopsies taken from human malignant mesothelioma express markers of osseous and cartilaginous differentiation (Donna & Betta, 1986; Yousem & Hochholzer, 1987; Andrion *et al.* 1989; Kiyozuka *et al.* 1999). In different experimental models, bone and cartilage were found in peritoneal malignant mesotheliomas that were induced by intraperitoneal injection of asbestos fibers (Rittinghausen *et al.* 1992). Interestingly, Fadare *et al.* 2002 found evidence of cartilaginous differentiation in human peritoneal tissue biopsies not related to any neoplasic process (Fadare *et al.* 2002). The high frequency of mesenteric heterotopic ossification (serous metaplasia) and pericardial calcification (Lemeshev *et al.* 1983; Yannopoulos *et al.* 1992; Wilson *et al.* 1999) could be related to an osteogenic and chondrogenic ability of adult mesothelial cells.

Several experimental models suggest that mesothelial cells may also form skeletal muscle. In fact, during the healing phase of a chemical-induced peritonitis, skeletal muscle fibers were found to develop de novo in the peritoneal lining of the adult rat diaphragm. The location and orientation of the fibers suggested an origin from mesothelial or submesothelial cells (Levine and Saltzman, 1994; Drakontides *et al.* 1999)

On the other hand, human adult mesothelial cells have shown inability to differentiate into endothelial cells. Although human epicardial cells can be induced to establish tubular network of the proper substrate resembling those formed by endothelial cells, some authors have reported the inability of these cells to express specific endothelial markers (van Tuyn *et al.* 2007)

It is possible that some of the embryonic epicardial-myocardial interactions are also maintained in adults. Coculture of adult rat cardiomyocytes with epicardial cells improves the maintaining of the differentiated phenotype (Eid *et al.* 1992). Conditioned medium of adult epicardium culture seems to protect the ischemic myocardium in mice (Zhou *et al.* 2011). Several authors have described the activation of adult epicardial cells after a myocardial infarction. Epicardial cells activate Notch signaling and migrate into the myocardium, giving rise to myofibroblastic cells, and acquiring some properties of mesenchymal stem cells (van Tuyn *et al.* 2007; Gittenberger de Groot *et al.* 2010; Winters *et al.* 2012). This process mimics the embryonic generation of EPDCs although the adult EPDCs do not differentiate into endothelium or cardiomyocytes (Zhou *et al.* 2012). However, priming of adult EPDC with the

actin binding protein Thymosin-β4 has been claimed to improve the regenerative and embryonic-like potential of adult EPDC after myocardial infarction (Smart *et al.* 2007; Smart *et al.* 2010). Nevertheless, these results have been questioned by other authors (Zhou *et al.* 2012; Banerjee *et al.* 2012). Thus, it is likely that most of the beneficial effects of adult EPDCs after myocardial infarction can be attributed to a paracrine signaling mechanism and not to a direct regenerative potential of the damaged heart. The same applies for non mammalian models such as the zebrafish. During myocardial regeneration in these species, after injury and removal of a part of the heart, the adult epicardium undergoes EMT and the resulting cells proliferate giving rise to a population of mesenchymal cells that do not differentiate in new cardiomyocytes but secrete proteins which promote angiogenesis and cardiomyocyte proliferation, thus contributing to the complete regeneration of the heart (Jopling *et al.* 2010; Kikuchi *et al.* 2010; Zhou *et al.* 2011; González-Rosa *et al.* 2012, Wang *et al.* 2013).

Conclusions and future directions

The embryonic coelomic epithelium appears as a highly active tissue and a main source of mesenchymal cells which can differentiate into a wide spectrum of cells types, mainly fibroblasts and smooth muscle, but also organ-spceific cells with very specialized physiological functions. This allows to complete the initial mesenchymal population that some organ primordia have from their inception, and in some cases (e.g. the heart or the gonads) the development of the organ itself is dependent of its invasion by CEDC. Despite the large number of papers dealing with this topic, a number of questions remain, for example:

- The map of the CEDC lineage. We still do not know what the contribution of CEDC to the stroma of organs such as the metanephros, spleen or pancreas is. Combination of data obtained from different Cre-drivers will be required to complete the map of the CEDC.

- Are there common mechanisms of EMT for all the processes of CEDC generation? The canonical EMT process involves the activity of genes of the Snail family of transcription factors, but we do not know how different processes of generation of CEDC are regulated at the molecular level.

- What factors are involved in the signaling properties of CEDC? Are there common signaling factors in all the organs invaded by CEDC?

- Are CEDC strictly pluripotential? Can different cell types be clonally derived from a single CEDC?

- How is CEDC differentiation regulated? Is this differentiation autonomous or is it dependent on local cues?

We are aware that the answers to these questions will have relevance from the clinical point of view. CEDC give rise to many lineages in normal development and they also may play physiological or physiopathological roles in adults. An understanding of how this happens can have a big impact in the treatment of many fibrotic diseases.

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FIGURE LEGENDS

Figure 1. Development of the coelomic epithelium. **A.** Chick embryo, HH10. A coelomic space appears in the lateral plate mesoderm, which is splitting in somatopleura (SOPL) and splanchnopleura (SPPL). ECT: ectoderm; EN: endoderm; NC: notochord; NP: neural plate; SOM: somite. **B,C**. In more anterior areas of the same embryo, splanchnopleura and somatopleura are giving rise to mesenchymal cells (arrowheads) that populate the space existing between the lateral plate mesoderm and the endoderm and ectoderm. Note that the primitive heart tube (HT) is continuous with the splanchnopleura. The insert shows the area enlarged in B (NT: neural tube). The endoderm of the anterior intestinal portal is located over the mesoderm due to the folding of this part of the body. **D.** The coelomic epithelium directly derives from the splanchnopleura and somatopleura when these cellular layers acquire epithelial features. However, the embryonic coelomic epithelium is activated in specific areas giving rise to mesenchymal cells. In this HH17 chick embryo, we can see signs of activation of the coelomic epithelium and generation of mesenchyme in the area lateral to the mesentery in which the adrenogonadal primordium will develop (arrowheads). In contrast, other areas of the coelomic epithelium show quiescence of their cells (arrows). The insert shows the area enlarged in D. **E.** Cartoon depicting different instances of coelomic epithelium derived cells (CEDC) generation. The coelomic epithelium (in red) gives rise to mesenchymal cells diffusely throughout the body wall, coelomic septa and splanchnopleural mesoderm, but this process is more active in areas where endodermal (eg. lungs, liver) and mesodermal organs (eg. adrenals, gonads) are developing. The heart is a special case since it is continuous with the splanchnopleura. In this case, a proliferation of cells over the septum transversum (the proepicardium) originates cells, which are transferred to the heart surface (1) and form the epicardium. Later, the epicardium (2) gives rise to mesenchymal cells which populate the heart and contribute to a part of the cardiac vascular and connective tissue.

Figure 2. Techniques for the study of the epithelial-mesenchymal transition of the coelomic epithelium. **A.** Morphological evidence combined with genetic lineage tracing. Cells derived from the epicardium (EP) can be seen delaminating from the surface of the heart and populating the heart wall. The G2-GATA4^{Cre};R26R^{YFP} mouse line allows for permanent fluorescent labeling of the embryonic epicardium and the cells derived from it. **B.** The intermediate filaments of the coelomic epithelium are constituted of cytokeratins (CK). During the epithelial-mesenchymal transition of this tissue the cytokeratin remains condense and transiently appear as perinuclear immunoreactive spots (arrowheads), as we can see in the digestive tract (DT) of this Wt1^{Cre};R26R^{YFP} E10.5 embryo. CE: coelomic epithelium. **C,D.** Direct labeling of the coelomic epithelium of quail embryos with CCFSE, a fluorescent marker. After three hours of injection in the

coelomic cavity epithelial cells of the dorsal mesentery (DM) and aorta (AO) appear labelled and show signs of migration (arrows). After 24 hours mesenchymal cells appear labeled (arrow) and some of them express the quail endothelial marker QH1 (arrowhead). **E-G.** Validation of the Wt1-Cre model for lineage tracing of the coelomic epithelium derived cells. Wt1 is expressed only in the coelomic epithelium of the developing lung as shown by colocalization of Wt1 protein with GFP which reports Wt1 promoter activation (arrows in E). Expression of both markers is high in the pleural wall (PW). Wt1-lineage cells, not expressing Wt1, appear in the stroma of the lung bud by the stage E11.5 (F). Some of these cells have differentiated into endothelial cells by E14.5, as demonstrated by the colocalization with the endothelial marker CD31 (G).

Figure 3. Derivatives of the CEDC in different organs. **A.** The G2-GATA4^{Cre};R26R^{YFP} mouse line allows for tracing of the epicardial-derived cells (EPDC) by the stage E11.5. Note how Wt1 is down-regulated in the cells migrating from the epicardium (EP) (arrows). Some EPDC can be seen into the ventricular wall (arrowhead). A: atrium, V: ventricle. **B.** EPDC accumulate into the atrioventricular groove and can be found into the ventricular myocardium by E14.5. **C.** In this E18.5 embryo, cells from the Wt1 lineage can be seen in the endothelium (arrowhead), smooth muscle (arrows) and perivascular areas of this coronary artery (CA). **D.** As reported by Rinkevich *et al.* (2012), most of the aortic smooth muscle of the trunk originates from CEDC. Our lineage tracing system allows also identification of endothelial cells with the same origin in the aorta (AO) of this E12.5 embryo (arrows). OE: oesophagous; LB: lung bud. **E**. The pleuroperitoneal folds (PPF) which are critical from diaphragm development also are constituted by CEDC. Note the strong expression of Wt1 in the coelomic epithelium and its downregulation in the PPF mesenchyme of an E11.5 embryo. LI: liver. **F.** The retinoic acid synthesizing enzyme Raldh2 is highly expressed in the coelomic epithelium by the stage E10.5, mainly in areas where activation on this epithelium is evident and gives rise to mesenchymal cells (arrows).

Figure 4. Derivatives of the CEDC in different organs. **A.** General view of the distribution of the G2-GATA4 lineage cells in an E13.5 embryo. Most of these cells are CEDC, and they appear in the liver (LI), body wall (BW), around the oesophagous (OE) and aorta (AO). These cells are very abundant in the adrenals (AD) and genital ridge (GR) but they are much scarcer in the nephric ridge (NR). **B.** Cells derived from the coelomic epithelium (CE) of the liver contribute to the endothelium of the sinusoids (SIN) (arrow) in an E16.5 embryo. Note the signs of migration from the coelomic epithelium (arrowheads). **C**. Interstitial Cells of Cajal (ICC) can be identified in the gastrointestinal tract by c-Kit expression. Some of these cells are of the Wt1-lineage (arrows). Newborn mouse. EN: endoderm.