Mesothelial to mesenchyme transition as a major developmental and pathological player in trunk organs and their cavities

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The internal organs embedded in the cavities are lined by an epithelial monolayer termed the mesothelium. The mesothelium is increasingly implicated in driving various internal organ pathologies, as many of the normal embryonic developmental pathways acting in mesothelial cells, such as those regulating epithelial-to-mesenchymal transition, also drive disease progression in adult life. Here, we summarize observations from different animal models and organ systems that collectively point toward a central role of epithelial-to-mesenchymal transition in driving tissue fibrosis, acute scarring, and cancer metastasis. Thus, drugs targeting pathways of mesothelium’s transition may have broad therapeutic benefits in patients suffering from these diseases.
Our internal organs and cavities are lined by a single continuous layer of epithelial cells known as the mesothelium that is derived from the embryonic mesoderm. It is the largest epithelial organ in the adult mammalian body. The mesothelium covers several body cavities and their internal organs, including (but not limited to) the pericardium, enclosing the heart, the pleural cavity, encasing the lungs, and the mesentery and peritoneum, encasing the various abdominal organs. The mesothelium also surrounds the internal reproductive organs in both males and females. The mesothelial membranes consist of a parietal (lining the body wall) and visceral (lining the internal organs) layer. The space in-between is filled with fluid, which acts to accommodate organ movement and decrease friction. Other well-known functions include protection against bacterial infections and their dissemination within the cavities, and to provide direct passage between the cavities and the internal organs (Box 1).

All vertebrate animals have a coelomic cavity that separates the outer and inner components of the body. It is formed as a result of a binary division of the lateral plate mesoderm. Through this division, the coelom is lined by two different, but continuous tissue components. During organ development, a cell population within these tissues acquires epithelial features, baso-apical polarization, and a basal lamina. It is at this point that we refer to these cells as the coelomic epithelium, the embryonic precursor of adult mesothelium. The coelomic epithelium significantly contributes to organ development, in particular by undergoing epithelial-to-mesenchymal transition (EMT). Through EMT, the coelomic epithelium contributes various cell types into the developing and growing organs, including fibroblastic cells and smooth muscle cells. We and others have identified some of its distinguishing markers, and used it to lineage trace the mesothelium’s embryonic and adult precursors. A subset of these cells are the likely self-renewing stem and progenitors of the underlying organ fibroblasts and smooth muscle. In adult life, the same pathways that are involved in EMT during organ development and growth reappear during injury and organ disease, such as infections, ischaemia, fibrosis (developing within organs), adhesions (developing in-between organs, tethering them to one another), and cancer. We propose here that trunk organ disease may be seen as the result of impaired EMT16. In line with this, epicardial Wt1 deletion in the epicardium develop peripheral oedema, pericardial haemorrhage, and heart wall thinning resulting from impaired EMT16. In line with this, epicardial Wt1 knockout mice show reduced expression of the key EMT activator Snail and Slug, and show reduced expression of the mesenchymal marker Vimentin. This is paralleled by increased expression of the epithelial markers E-cadherin and cytokeratin17. Wt1 is an upstream regulator of the canonical

**Mesothelial cell EMT involvement in healthy organ development and growth**

**Heart development.** The coelomic epithelium that covers the developing heart is derived from a group of progenitor cells near the venous pole of the heart known as the proepicardial organ, which in turn comes from a thick mass of cranial mesenchyme called the septum transversum. The precursors of the coelomic epithelium migrate to form a layer of tissue between the pericardium and the heart, termed epicardium. A subpopulation of these cells proliferates and undergoes EMT that allows them to invade the developing heart and colonize its internal spaces as subendocardial fibroblasts, and by encircling the developing coronary blood vessels, where they develop into smooth muscle cells (Fig. 1). Indeed, several independent lineage-tracing experiments using epicardial-specific Cre-LoxP transgenes, e.g., mesothelin (Msln), T-box transcription factor 18 (Tbx18), and Wilms’ tumour 1 (Wt1), have demonstrated that epicardial cells can differentiate into coronary smooth muscle cells, and fibroblasts (including pericytes). The importance of the epicardium in heart development has been further demonstrated with epicardial knockout models. From these studies, a number of genes have been identified that highlight epicardial EMT as a crucial factor in heart morphogenesis. TBX18 over-expression promotes EMT in cultured epicardial cells by inducing the expression of Slug14. The role of the transcription factor Wt1 on epicardial development has been described in more detail. Wt1 binds directly to the Snail and E-cadherin promoter sites, thereby either promoting or repressing transcription respectively. Mice with a Wt1 deletion in the epicardium develop peripheral oedema, pericardial haemorrhage, and heart wall thinning resulting from impaired EMT16. In line with this, epicardial Wt1 knockout mice have reduced epicardial expression of the key EMT activators Snail and Slug, and show reduced expression of the mesenchymal marker Vimentin. This is paralleled by increased expression of the epithelial markers E-cadherin and cytokeratin17.
Fig. 1 Mesothelial involvement in embryonic development. Coelomic epithelial cells provide the bulk of fibroblastic and smooth muscle lineages of the body and critically support EMT during embryonic development of different organs. A common set of genes, in particular WT1, TBX18, MSLN, Notch1, GATA4, and their downstream effectors underlie these EMT programmes to regulate mesenchymal differentiation. Four examples are given: (1) heart, (2) liver, (3) gonads, and (4) the lungs, showing how the coelomic epithelium expands and ingresses to form tissue-specific fibroblasts and smooth muscle lineages (displayed as purple) by virtue of EMT. For further details, see the main body of text.
WNT signalling pathway that leads to activation of β-catenin. Epicardial-specific β-catenin-null mice have disturbed epicardial EMT, blunted invasion of the heart muscle, failed expansion of the sub-epicardial space, and impaired differentiation of epicardial cells into coronary smooth muscle. EMTC also fails in cardiac fibroblasts (that remain in the epicardium) in the absence of the β-catenin-binding partner TCF21. Another WNT protein, WNT5A, has also been implicated as an epicardial factor that promotes compact heart muscle growth. Both Wnt5a and Wt1 mouse knockout hearts have thin compact heart muscle. Another epicardial gene whose expression is dependent on WT1 and that is important for heart development is retinaldehyde dehydrogenase 2 (Raldh2), encoding the enzyme that controls retinoic acid availability. Wt1 knockouts show reduced Raldh2 expression, and like Wt1 knockout embryos, Raldh2-/- embryos, treated with exogenous retinoic acid to bypass early embryonic lethality, exhibit myocardial hypoplasia and coronary vessel abnormalities. Retinoic acid supplementation also works to restore embryonic EMT and viability of Wt1 knockout hearts, suggesting a functional link between WT1 and Raldh2.

EMT is controlled by a plethora of factors, where WT1 appears to depend on the stage of development. Collectively, epicardial EMT, and to ingress and form a cluster of SF1+ gonadal somatic precursor cells, which later give rise to the supporting Sertoli cells of the testis cords and seminiferous tubules, or the follicular (or granulosa) cells of the ovarian follicles. The supporting cells are not the only cells derived from the coelomic epithelium in the primitive gonads of mice. Cell-tracking experiments have shown that the SF1+ gonadal precursor cells generate the testicular peritubular myoid cells (PMCs, the smooth muscle cells surrounding the seminiferous tubules in the testis) through EMT and the steroidogenic Leydig cells and Theca cells in testis and ovaries, respectively. The molecular mechanisms driving coelomic epithelium transformation into the genital ridge have not been laid out as comprehensively as for the embryonic heart or liver, but a similar gene signature in gonadal tissues can be observed around this time. Like the heart and liver, in the developing gonads Wt1, Gata4, Tcf21 and Tbx18 are one of the earliest activated genes, present throughout the whole urogenital ridge. Their expression is quickly followed by Sf1, which remains restricted to the genital ridge. Tbx18 null mice appear to have defective EMT in that they develop ectopic patches of fibroblasts along the developing gonads, and Tcf21 knockouts lack a distinct mesenchymal compartment. GATA4 is thought to be most important in the undifferentiated precursor population giving rise to the genital ridge. Gata4 null mice do not develop a genital ridge at all, and the expression of Sf1, but not WT1, is significantly decreased. Similarly, Fog2 mutants have abnormal gonads through impaired thickening of the genital ridge. Mice lacking functional WT1 initiate gonad formation by thickening of the genital ridge. Consequently, disruption of Sf1 expression results in delayed and decreased EMT, disrupted cell development of the reproductive (gonadal) system. The adrenal cortex and gonads share a common developmental origin, the adrenogonadal primordium. In mice, the adrenogonadal primordium can be first detected as a thickening of the coelomic epithelium at approximately E9.5 (refs. 42,43). By E10.5, the adrenogonadal primordium splits into the adrenal and gonadal primordia that will continue to differentiate separately. The gonadal primordium, also known as the genital ridge, is initially bi-potential, and begins development through the increased proliferation of the coelomic epithelium (Fig. 1). This increased proliferation leads to the transformation of the monolayer epithelium into a dense and pseudo-stratified layer. Lineage-tracing studies using cell-permeable dyes or genetic Cre lines (Wt1, Tbx18, Sf1) have shown that following these events, the basement membrane underneath the coelomic epithelium disintegrates, which allows the proliferating cells of the coelomic epithelium to undergo EMT, and to ingress and form a cluster of SF1+ gonadal somatic precursor cells, which later give rise to the supporting Sertoli cells of the testis cords and seminiferous tubules, or the follicular (or granulosa) cells of the ovarian follicles. The supporting cells are not the only cells derived from the coelomic epithelium in the primitive gonads of mice. 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Tbx18 null mice appear to have defective EMT in that they develop ectopic patches of fibroblasts along the developing gonads, and Tcf21 knockouts lack a distinct mesenchymal compartment. GATA4 is thought to be most important in the undifferentiated precursor population giving rise to the genital ridge. Gata4 null mice do not develop a genital ridge at all, and the expression of Sf1, but not WT1, is significantly decreased. Similarly, Fog2 mutants have abnormal gonads through impaired thickening of the genital ridge. Mice lacking functional WT1 initiate gonad formation by thickening of the coelomic epithelium through active division, but the genital ridge quickly regresses before it is completely formed. There is further evidence to suggest that WT1 regulates Sf1 in the undifferentiated genital ridge. Consequently, disruption of Sf1 expression results in delayed and decreased EMT, disrupted cell
ingression during the formation of the genital ridges, and consequently fewer SF1 + gonadal precursor cells and gonad size. A microarray screen for WT1 wildtype and knockout E11.0 embryos highlighted Mac16 as the top gene with regard to the coelomic epithelium, which was completely absent in knockout tissues. Mucin 16 is highly expressed in mesothelial cancers, and has been shown to promote EMT, in part through interaction with MSLN. Thus, similar to heart and liver development, WT1 appears to regulate EMT in the developing gonads, in part through SF1, while GATA4 closely cooperates alongside WT1 to drive initial formation of the genital ridge. In XY mice, knocking out WT1 after sex determination results in disruption of developing seminiferous tubules through the aberrant differentiation and development of Sertoli cells. Sertoli-specific WT1 mutants also show abnormal PMC and Leydig development. Interestingly, the precursors of Sertoli cells express only very low amounts of SF1, despite a high abundance of WT1, and it has been shown that after sex determination, SF1 is repressed by WT1 through direct binding to the promoter region. In the absence of SF1, many gene pathways are shared between these organs. WT1, -catenin, Notch1, and GATA4, at some stage in lung development, are all selectively expressed in coelomic epithelial cells that are involved in several types of heart diseases, including myocardial infarction in mice results in re-activation of the epicardium and the re-expression of several embryonic epicardial genes, such as Raldh2, Wt1, Gata4, and Msh2. The re-expression of embryonic genes is typically confined to the lesion site, where the epicardium progressively expands and undergoes EMT to generate cardiac fibroblasts within the vicinity of the infarct. These areas are rich in expression of mesenchymal cell type markers, including fibroblast-specific protein 1, pro-collagen I, collagen III, fibronectin, α-SMA, SM-22α, and smooth muscle myosin heavy chain, indicative of fibroblasts that are actively depositing extracellular matrix proteins. Epicardium-specific inhibition of these coelomic epithelial genes significantly diminishes cardiac fibrosis. For example, Wt1Cre;Ctnmb1fl mice are null for β-catenin expression in the epicardium, and show minimal signs of epicardial expansion and EMT when subjected cause lung lobulation abnormalities, which can be attributed to growth delay and abnormal branching morphogenesis. Internally, Gata4 mutants show dilated distal airways and patches of thickened mesenchyme, characterized as ectopic expression of α-SMA in the mesothelial and submesothelial layer. Similar observations have not been made in the pleura of mice harbouring a mutant of P300, another known co-factor for GATA4. These results suggest defects in GATA4 expression perturb coelomic epithelium-driven EMT during lung morphogenesis, presumably through impaired signalling from the mesothelium to the underlying endoderm. Similarly, coelomic epithelium-selective (Dermo1+) β-catenin mutants show impaired lung growth as early as E12.5, primarily through reduced mesenchymal expansion, and defective Notch signalling in Dermo1+ RBFk mutants results in impaired vascular, but not bronchial smooth muscle differentiation. Systemic WT1 knockout embryos show severe growth defects in the lungs, displaying an irregular rounded-shape and abnormally fused lobes. Raldh2 mutant mice or lung explants treated with the pan-RXR antagonist BMS493 have perturbed lung bud formation. Wt1 and Raldh2 mutants initially form a pleuropulmonary cord, but do not fuse with the coelomic epithelial lining of the lung hilus, and Tbx18 knockout mice fail to develop the pleuropulmonary membranes at all. In this regard, drivers of EMT have been shown to be essential in the underlying pathology. WT1 knockout mice maintain an intact basal lamina under the pulmonary coelomic epithelium, and show strongly reduced vimentin immunoreactive cells in the lung mesenchyme, indicative of impaired mesothelial EMT. Several downstream targets have been shown elementary in this process. Embryonic lung explants exposed to CCSFE to label pleural coelomic epithelium show extensive EMT after 48 h, which can be further controlled through TGF-β or Notch signalling modulation. Another putative regulator of mesothelial EMT is Enhancer of zeste homologue 2 (EZH2), a histone methyltransferase that in mesothelial cancers is known to repress EMT. Wt1-dependent EZH2 knockouts display uncontrolled EMT in the coelomic epithelium, and develop multiple heart and lung abnormalities by generating ectopic patches of smooth muscle around E14.5, originating at the proximal end of lung lobes, and then spreading out distally around E18.5. These patches co-express smooth muscle protein 22 (SM-22α) and α-SMA, indicative of a smooth muscle phenotype, and are closely associated with WT1+ cells at the periphery of the lung.

Development of the lungs. In mouse embryos, the lung primordium emerges from the ventral foregut around E9.5, consisting of a simple tube of endodermal epithelium surrounded by splanchnic mesodermal mesenchyme that is lined by a coelomic epithelium layer. This tube then branches to form the pulmonary sacs. Anterior to the developing lungs are bilateral folds of tissue which extend from the body wall, known as the pleuropulmonary membranes, which grow towards the midline, each encapsulating a common cardinal vein and phrenic nerve. They are further stretched when the initially straight embryonic heart tube is transformed into a helical loop, to finally fuse with the root of the lungs to close off the remaining communication between the pericardial and pleural spaces around E13.5 (ref. 75) (Fig. 1). Because of the contiguous nature of the heart and pleura, many gene pathways are shared between these organs. WT1, TBX18, RALDH2, -catenin, Notch1, and GATA4, at some stage in lung development, are all selectively expressed in coelomic epithelial cells surrounding the lung buds or pleuropulmonary folds. Lineage-tracing studies have confirmed that by E11.5, WT1+, 78,79,82–84, MSLN+6 and Notch1+79 coelomic epithelium cells covering the lungs migrate inward to give rise to α-SMA+ fibroblast and smooth muscle cells that make up both arteries and veins in the vascular wall, or those positioned circumferentially around the airways. These results have been extended towards the postnatal lung using inductive Cre lines, indicating that the coelomic epithelium contributes to these lineages even after birth. Equally, WT1+ descendants from the pleuropulmonary folds generate the mesenchyme surrounding the cardinal veins adjacent to the growing lungs. Knockout studies have corroborated these findings. Defects in Gata4 or its co-factor Fg2
to ischaemia reperfusion injury\textsuperscript{101}. In mammals, expression of a dominant-negative CCAAT/enhancer binding protein, which in essence is upstream of and controls the transcription of \textit{Raldh2} and \textit{WT1} in adult mouse epicardium, can instil a cardioprotective effect and partially prevent myocardial fibrosis after ischaemia reperfusion\textsuperscript{102}. In spite of the fibrotic potential of injured mesothelium of the heart, its presence is probably required for normal wound repair. In adult zebrafish, a species with robust regenerative potential after injury, genetic depletion of the epicardium after experimentally resecting parts of the heart ventricle inhibits cardiomyocyte proliferation and delays muscle regeneration\textsuperscript{103,104}. Similarly, disruption of thymosin \beta\textsubscript{4}-mediated paracrine signalling towards the epicardium impairs smooth muscle cell development in mice, and its expression facilitates vascular repair following myocardial infarction\textsuperscript{105,106}. It is worth mentioning that not every cardiac injury model shows evidence for epicardial EMT, for example, in the pressure overload model through constriction of the ascending aorta\textsuperscript{107,108}. However, this model primarily results in perivascular fibrosis, with no signs of elevated WT1 or TBX18 expression in the epicardium\textsuperscript{109}, and thus likely originates from a different, possibly endothelial source\textsuperscript{107,108}.

\textbf{Pathology of the liver.} The mesothelium covering the liver is comprehensively involved in liver pathologies. Different hepatic injury models that exhibit varying degrees of fibrosis show varied involvements of the hepatic surface mesothelium, or hepatic stellate cells (HSCs), which are derived from mesothelium. In fact, different injury models show diverged severities of fibrosis that stem from the level of activation of the mesothelium and HSCs. Lineage-tracing studies have confirmed an active role of the surface mesothelium as the cell-of-origin of HSCs and myofibroblasts in response to carbon tetrachloride, which can be inhibited through antagonism of TGF-\beta\textsubscript{1} signalling\textsuperscript{110}. Similarly, activated portal fibroblast (the predominant source in cholestatic liver injury induced by bile duct ligation) exhibit a high expression of MSLN upon bile duct injury, further suggesting that these cells originate from a mesothelial source\textsuperscript{16,111}. Consequently, MSLN-deficient mice are less susceptible to liver fibrosis compared to wild-type mice\textsuperscript{112}, and conditional ablation of mesothelial-derived MSLN\textsuperscript{17} portal fibroblasts in bile duct-injured mice show significantly reduced liver fibrosis\textsuperscript{113}. The mechanism by which MSLN promotes fibrosis presumably acts through its antagonistic actions on a TGF\textsubscript{B}RI inhibitor complex called thymocyte antigen 1\textsuperscript{1} (ref. \textsuperscript{113}). Like the heart, the mesothelium also supports liver regeneration upon injury. The mesothelium-derived paracrine factors MKD and PTN facilitate hepatocyte growth following hepatectomy\textsuperscript{114,115}, analogous to the mesothelium’s stimulatory and regenerative role during intestinal crypt\textsuperscript{116} and embryonic heart regeneration\textsuperscript{117}. Conditional \textit{Gata4} knockout mice have a high degree of liver fibrosis when treated with subcutaneous injections of carbon tetrachloride, inducing hepatic toxic liver injury\textsuperscript{118}. Chronic exposure in \textit{Gata4}-deficient mice results in increased liver hydroxyproline content, infiltrating CD45\textsuperscript{+} cells, and serum alanine aminotransferase and aspartate aminotransferase levels compared to control livers\textsuperscript{119}, all indicative of fibrosis associated with progressive liver failure. These findings likely indicate that mesothelial expression of \textit{Gata4} inhibits liver fibrosis through its effects on lineage maintenance in Stellate cells.

\textbf{Repair of the (female) reproductive system.} The ovaries regenerate their surface mesothelium (also called ovarian surface epithelium, OSE) throughout postnatal life. Rupturing of the follicles that follows each ovulatory cycle is followed by complete regeneration of the mesothelium without fibrosis. In spite of this, ovulatory wound repair is nonetheless associated with mesothelial EMT that temporarily permits displaced OSE to assume a mesenchymal phenotype within the ovarian cortex and migrate actively towards the ovulatory wound\textsuperscript{118–121}. Additionally, the EMT regulator TGF-\beta\textsubscript{1} is highly expressed in the serosal fluid around the ovaries in response to ovulation, and significantly increases the sphere-forming efficiency of OSE progenitor cells, further suggesting EMT is a requisite for the maintenance of OSE integrity\textsuperscript{122}. It has been proposed that discrete regions within the OSE may serve as cell sources that replenish or close the ovulatory wounds\textsuperscript{121}, and several markers have been identified\textsuperscript{123,124}, including lymphocyte antigen 6 complex, locus A (LY6A), leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), and RALDH (WNT target genes). For example, a subset of the OSE expresses LGR5 that serves to maintain OSE homeostasis and act as a cell-of-origin for new OSE during the ovulatory cycle\textsuperscript{121,126}. The exact location of LGR5\textsuperscript{+} cells is still under debate, as to whether they are restricted to the ovary hilum\textsuperscript{121}, or are more widespread, and respond locally to rupturing follicles by actively proliferating\textsuperscript{126}. Nonetheless, these findings suggest that the activity of LGR5+ mesothelial cells are most likely stem cells that are tailored to meet the specific growth requirement of the local OSE. The integrity of LGR5+ cells is likely maintained by active WNT signalling, as these cells secrete WNT4 and express several WNT target genes such as TROY and inhibitor of DNA binding 2 (ref. \textsuperscript{126}). High RALDH expression has also been identified in the OSE transitional zone, which constitutes approximately 5% of the total OSE population. In vitro studies have shown this cell subset has higher capacities to generate cell clones as compared to RALDH- cells, and similar to LGR5+ cells, these cells exhibit a greater degree of proliferation after ovulation\textsuperscript{121}. It is unclear at this point whether these cells represent the same cell population, and importantly, whether these genes are involved in regulating mesothelial EMT to drive ovulatory wound repair.

\textbf{Pathology of the lungs.} There are two scarring pathologies of the lungs in which pleural mesothelium is known to be involved: pleural fibrosis (PF), and interstitial lung disease. PF manifests either as discrete localized fibrotic lesions, or more diffusely organized pleural fibrotic patches, which in some cases result in fusion of the pleural membranes (Fig. 2)\textsuperscript{127}. Both parietal and visceral layers can be affected, but is generally only clinically significant when it involves the visceral\textsuperscript{128}. Various etiologies can account for PF, from pleural effusions (e.g. rheumatoid, tuberculous, or uraemic pleurisy, bacterial empyema, retained hemorhorax), to particle or compound exposures (e.g. asbestos, carbon nanoparticles, medications) or secondary malignancies\textsuperscript{128}. Experimental models to study PF have relied mostly on variations of the bleomycin mouse model. In mice receiving intraperitoneal or intratracheal bleomycin, increased expression of the mesothelial markers calretinin and \textit{WT1} combined with TGF-\beta\textsubscript{1}Smad2/3 is observed in the pleura and underlying par enchyma of mice\textsuperscript{129,130}. Consequently, mice treated with the TGF-\betaRII targeting miRNA miR-18a-5p have reduced (sub-)PF after bleomycin administration\textsuperscript{131}. Strikingly, intratracheal bleo mycin combined with carbon nanoparticles results in a more persistent and severe pulmonary response, including adhesions and severe and progressive (sub-)PF, preceded by pleural thickening and inflammation\textsuperscript{132}. The engulfment of carbon nanoparticles by the mesothelium contributes to the severity of this model, driving rapid upregulation of \alpha-SMA and release of MMP-9 and TGF-\beta1 in the pleural fluids\textsuperscript{132,133}. A role for \beta-catenin signalling has been described in a model for pleural
effusion, a known risk factor for PF. Mice subjected to *Streptococcus pneumoniae* develop empyema accompanied by α-SMA+ expression in the pleura, pleural thickening and reduced lung function, which can be inhibited by GSK-3β antagonist 9ING41 (ref. 134). Interstitial lung diseases represent another collection of heterogeneous fibrotic parenchymal disorders, of which idiopathic pulmonary fibrosis (IPF) is a more clearly defined subset. In line with the mesothelium as the site of origin, clonal analyses in IPF lungs have revealed the presence of a polyclonal, rather than a monoclonal population of fibroblasts. This suggests fibrotic foci are due to a reactive process responsive to local environmental stimuli, rather than a single malignant neoplasm growing through the lung. These foci are part of a continuous fibrotic reticulum that extends from the mesothelial surface into the lung parenchyma, identifying the mesothelium as the most likely source of origin (Fig. 2)135. Further evidence has been derived from mathematical models, in which increased stiffness of the visceral pleura, e.g., following EMT and matrix synthesis, is predicted to be key in driving mechanotransduction pathways to propel a fibroblast activation chain towards the parenchyma 136. In support of this, the pleura and underlying parenchyma of human IPF patients show strong immuno-labelling for MSLN, WT1 or calretinin130,137,138, and when isolated and cultured display increased contractility and movement compared to healthy mesothelium130. The extent of labelling correlates positively with the degree of parenchymal fibrosis in patients137. Importantly, these markers are absent in other fibrotic diseases of the lung, such as chronic obstructive pulmonary disease or cystic fibrosis, in which the mesothelium plays no significant role. In vitro, pleural...
mesothelial EMT is controlled by a WT1–TGF-β1–Smad-2 axis, which is further supported by mouse models. Adenoviral transmission of intrapleural TGF-β1 induces progressive PF of the viscera and underlying parenchyma, with strong cytokarkin and α-SMA in both tissue compartments. Lineage tracing of these cells with WT1ΔGFP-Cre mice or GFP-labeleed pleural mesothelium injected in the pleural space, combined with intratracheal recombinant TGF-β1 show strong GFP and α-SMA co-labelling in the lung parenchyma underneath the pleura, indicating active migration of the mesothelium.

**Pathology of the abdominal wall (peritoneum).** Damage to mesothelium covering the three cavity walls or the internal organs induces the development of fibrosis on their surfaces that can attach to neighbouring healthy tissues, termed adhesions (Fig. 2). Adhesions are the most common side-effect of abdominal surgery that stems from mechanical trauma due to surgical tissue handling, ischaemia at incision sites, foreign bodies or tissue desiccation. As well as after surgery, adhesions can result from inflammatory processes, from infections, or as an adverse response to dialysis. Kidney dialysis takes advantage of the peritoneal surface mesothelium as a means of extravasation of plasma fluids from the blood. The dialysate solution, which contains solvents that are hyperosmotic, hyperglycaemic, and of low pH, gradually damages the exposed surface mesothelium and leads to its activation. Exposure of the mesothelium to dialysis solution increases mesothelial cell death, but also eventually leads to thickening of the mesothelium and generation of myofibroblasts and fibrous collagen bands at the injured surface. Once fibrous bands initiate at the surface, the mesothelium never reaches complete regeneration, even months after cessation of dialysis. More severe cases involve the development of encapsulating peritoneal sclerosis on the surface mesothelium that follows prolonged exposure to dialysis, chronic inflammation, or several other secondary acute intra-abdominal events. Similar to other mesothelial pathologies, the pathomechanism that leads to encapsulation is poorly understood. Organ encapsulation stems from thickening and fibrosis of the surface mesothelium and leads to a range of ailments, including obstruction and shortening of the bowels, abdominal pain, weight loss, malnutrition, sepsis, or death. The level of encapsulation and thickening directly correlates with the level of mesothelial activation, suggestive of a pathomechanism wherein mesothelial cells undergo varied extents of EMT in response to durations of dialysis. Human peritoneal–intestinal adhesion tissues resulting from abdominal surgery show strong immunolabelling for cytokerin, calretinin, WT1, and α-SMA in the mesothelium and surrounding fibroblasts, suggesting these fibroblasts derive from mesothelial cells that have undergone EMT. The advent of lineage tracing has confirmed these observations. Although WT1Δ+ mesothelial cells have been reported to proliferate and actively repair the peritoneal surface upon injury, various models of peritoneal fibrosis (i.e. hypochlorite, daily intraperitoneal injection of dialysate or mouse TGF-β adenoviral administration) have reported a mesothelial and submesothelial origin for myofibroblasts in peritoneal fibrosis. Mechanistic studies have suggested TGF-β to be an active player in peritoneal EMT and cell culture studies have indicated MSLN is involved in driving cancer EMT. Mesothelial-derived cancers are remarkably heterogeneous. Most metastasizing solid tumours must first invade the tumour stroma and access the vasculature. Therefore, an early EMT is necessary in order to adopt a motile, invasive phenotype. However, the unique trans-coelomic route of mesothelial-derived cancer cells creates an exceptional microenvironment. In contrast to most solid tumours, mesothelium-derived cancer cells exfoliate directly into the peritoneal cavity. Thus, early events in metastatic dissemination do not necessarily require a mesenchymal phenotype. As a result, both pleural and ovarian mesothelial cancer cells can display either epithelial or mesenchymal morphologies, or exist as an intermediate state. For example, sarcomatoid mesotheliomas resemble spindle-shaped cells that mimics the morphology of mesenchymal tumours, whereas epithelioid mesotheliomas represent a more epithelial-
Exosomes may contain growth factors used to directly modify the cancer microenvironment rather than being a consequence of it. Moreover, the similarities in gene expression programmes across diverse organ systems and disease states suggest common roles of the mesothelium in driving organ pathophysiology. A deeper understanding of trunk organ diseases could thus emerge from studying the mechanisms underlying embryonic mesothelial EMT. Such a developmental focus might also identify much-needed new therapeutic avenues for trunk organ disease. For further research, three main aspects in mesothelium’s biology are particularly relevant: (1) intermediate stages of EMT, (2) common and organ-unique mechanisms of EMT, and (3) mesothelium’s cell type heterogeneity and their relation with EMT.

Intermediate stages of EMT. Coelomic epithelium cells of the proepicardial organ need to go through a series of highly orchestrated steps to populate the embryonic heart primordium: (1) directed migration towards the heart surface, (2) controlled proliferation to increase cell numbers, (3) migration towards the inner compartments of the heart, and (4) gain of mesenchymal traits to adopt fibroblastic and smooth muscle characteristics. Not only do these stepwise events require alternating commitments to EMT and MET to sustain epicardial identity while also providing the mesenchymal lineages of the heart, they also require intermediate stages within the EMT spectrum. The plethora of genes described here that regulates these events, and the disparity between knockout lines with regard to their effects on EMT, highlights the complexity of (different stages of) EMT and the gene regulatory networks that are at play. Hence, it is important to consider that during scarring and wound healing in adult life, the same extent of heterogeneity may be expected, where cells may linger in intermediary stages between a full epithelial and mesenchymal state to promote wound healing or scarring. For example, studies on mesothelial ovarian cancers revealed they could be grouped in different compositions along the EMT spectrum178,199,200, which may reflect differently on their ability to migrate, adhere, invade or survive.201 This stresses the importance to focus on the entire range of EMT to allow for a more dynamic interpretation of the fluidity and plasticity of this cell state. It may also provide more insight into the different scarring signatures recognized among mesothelial pathologies, e.g. why is the viscera sometimes more affected than the parietal surface, and why do some pathologies involve fibrous scars to exclusively develop on organ surfaces, whereas others extend into the parenchyma?7

Mechanisms of known EMT drivers. At present there is an underestimation of the complexity of WT1 signal regulation, and the networks in which it functions. This complexity includes alternative start codons, splice sites, and RNA editing that can theoretically give rise to 36 different proteins and many more potential dimers.202 The creation of mice ablated for specific WT1 isoforms has provided direct evidence that WT1 splicing variants perform distinct functions in embryonic development45,203. Moreover, there may be gene elements that can influence the effects of WT1 isoforms. For example, WT1 drives EMT in the coelomic epithelium covering the heart, liver, and lung primordium through close cooperation with GATA4 under control of the G2 enhancer domain.204, Contrary, in kidney development WT1 maintains epithelial integrity, where the Gata4 gene is not driven by the G2 enhancer.205 Thus, much work remains on the exact mechanisms that control mesothelial EMT, which likely requires a more systems-based approach. Establishing such gene regulatory networks and feedback loops will further our chance of developing successful therapies to reduce scarring and prevent fibrosis.
Cell heterogeneity within the mesothelium. Single-cell transcriptomic studies have identified at least three epicardial cell sub-
sets in the healthy adult heart of zebrafish and a sub-population of c-kit+ cells in the adult mouse epicardium. However, although cultured epicardial cells from human and mouse adults are able to recapitulate at least part of the differentiation potential of their embryonic counterparts, isolated adult epicardial cells of mice are not able to generate colony-forming units ex vivo. As such, if the adult mesothelium of mice and humans contains a sub-population of cells with stem-like properties, they may exist in a dormant state. A study in mice has recently revealed the presence of epicardial cell heterogeneity after subjecting hearts to models of myocardial infarction. These cells expressed the progenitor markers CD90+, CD44+, CD29+, c-kit+, but were distinct from their embryonic counterparts. Rather than an existing dormant progenitor state, stemness may be conferred to differentiated cells by changes in the local microenvironment in response to tissue injury. The idea that cell reprogramming can generate facultative stem cells emerged in the local microenvironment in response to tissue injury. The idea that cell reprogramming can generate facultative stem cells emerged in the local microenvironment in response to tissue injury.

In recent years there has been a proliferation of studies on drugs targeting WT1 and MSLN, many of which are in advanced stages of clinical testing. Drugs include recombinant immunotoxin proteins, vaccines, monoclonal antibodies, and antibody-drug conjugates. One example is Amatuximab, a chimeric high-affinity monoclonal IgG1/k antibody targeting MSLN, which elicits cellular toxicity against MSLN-expressing tumour cells, and reduces invasive capacity and tumour sphere formation. Amatuximab improved median overall survival and was well tolerated in phase II clinical trials of patients with advanced pleural mesothelioma. It would be important to determine whether patients receiving therapies targeting MSLN and WT1 have equally reduced risks of developing additional diseases such as ischaemia, chronic fibrosis, and adhesions. In this regard, other embryonic markers, such as WNT effectors, β-catenin, Notch1, and RALDH2, some of which are highly expressed in mesothelial cancers similarly warrant further investigation. Clinical studies that target mesothelial cancers may help clarify the extent of co-morbidity with other mesothelial pathologies where EMT plays an important role, opening new clinical avenues through which our current understanding of the EMT programme and transitional events can be exploited and organ disease can be curtailed.

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Additional information

Competing interests: The authors declare no competing interests.

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