Int. J. Dev. Biol. 45 (S1): S143-S144 (2001)

Short Report

Origin of endothelial cells from mesothelial-derived mesenchymal cells in the liver of avian embryos

RAMÓN MUÑOZ-CHÁPULI*[1], RITA CARMONA, MAURICIO GONZÁLEZ-IRIARTE, JOSÉ MARÍA PÉREZ-POMARES[2], DAVID MACÍAS, GERARDO ATENCIA and MARÍA JOSÉ ARANDA

[1]Departamento de Biología Animal, Facultad de Ciencias, Universidad de Málaga, Spain and
[2]Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, USA

ABSTRACT We have tested the hypothesis of an origin of mesenchyme from the coelomic mesothelium of the developing avian liver and the differentiation of sinusoidal endothelial cells from mesothelial-derived mesenchymal cells. Our results have shown that 1) Mesothelial cells of the developing liver and the submesothelial mesenchymal cells are simultaneously cytokeratin (CK) and vimentin (VIM) immunoreactive between HH18 and HH28. 2) A number of early sinusoidal cells are also CK immunoreactive. In the quail, CK colocalizes with the vascular marker QH1 in these cells. 3) In late embryos (>HH30), only the mesothelium remains CK+. 4) Mesothelial and submesothelial mesenchymal cells express the transcription factors Slug and WT1, both related to the epithelial-mesenchymal transition. 5) When the surface mesothelium was stained *in vivo* with CCFSE, a carboxyfluorescein derivative, isolated sinusoidal cells were labelled after 24-48 h. These observations suggest that mesenchymal cells originate from the coelomic mesothelium of the developing liver and differentate into endothelial cells of the hepatic sinusoids.

The vascularization of the vertebrate embryo occurs through two processes, vasculogenesis, the formation of the primary capillary plexus from mesodermal progenitors, and angiogenesis, the growth of vessels from preexisting ones (Risau and Flamme, 1995). Thus, vasculogenesis gives rise to the earliest vascular network while the later formation of vessels in the developing organs depends either from further events of vasculogenesis or from ingrowth of vessels into the newly formed organs.

The liver forms from an outgrowth of the embryonic endoderm and it is primarily vascularized through vasculogenesis (Sherer, 1991). The proliferating hepatocytes intermingle with angioblasts which do not coalesce in a capillary plexus, as it occurs in other organs, but in a wide system of endothelial-lined cavities called the hepatic sinusoids. The origin of the liver angioblasts is uncertain. In this report we show the results of a study about the origin of the hepatic angioblasts in the avian embryo. We suggest that at least a part of these angioblasts derive from cells originating from epithelial-mesenchymal transformation (EMT) of the liver mesothelium. This evidence supports our hypothesis about the localized origin of pluripotential cells from embryonic mesothelial cells (Muñoz-Chápuli *et al.,* 1999).

We have checked, in the mesothelial and submesothelial cells of the liver of quail and chick embryos, the presence of markers of EMT. First of all, we have immunolocated the intermediate filaments cytokeratin (CK) and vimentin (VIM), which are epithelial and mesenchymal markers, respectively, and transiently colocalize in epithelia fated to transform in mesenchyme as well as in the early epithelial-derived mesenchyme (Pérez Pomares *et al.,* 1997, 1998). We have also immunolocated the zinc-finger transcription factors, related to the EMT, Slug (Nieto *et al.,* 1994) and WT-1 (Moore *et al.,* 1999). On the other hand, we have labelled *in vivo* the liver mesothelium with the fluorescent marker CCFSE, a carboxyfluorescein derivative which becomes fluorescent when it is incorporated to the cells and stands up formaldehyde fixation and wax embedding (Sun *et al.,* 2000)

The mesothelium of the liver, as well as the underlying mesenchyme, expresses all the markers of EMT during the period of maximum growth of the liver, when the sinusoids are forming, i.e. from HH18 to HH27. By these stages, the mesothelial cells and the underlying mesenchymal cells are CK+/VIM+ (Fig. 1 A,B) and express the transcription factors Slug and WT-1 (Fig. 1 F,G). Slug is expressed by the mesenchymal cells throughout the volume of the liver, while the expression of WT1 is restricted to the most superficial areas.

Interestingly, a number of hepatic sinusoidal cells by these stages are CK+, a conspicuous difference with other embryonic endothelial cells (Fig. 1 A,D). In the quail embryo, cytokeratin colocalized with the endothelial marker QH1 in the sinusoidal walls, especially in the marginal areas where new sinusoids were assembling (Fig. 1 C,D). However, in later embryos (>HH30), CK immunoreactivity is found only in the coelomic mesothelium (Fig. 1E).

When we labelled with CCFSE the liver surface, we recovered the label after 24-48 h in mesothelial cells, some mesenchymal cells and in isolated sinusoidal cells (Fig. 1H). We do not think that the staining can be due to the penetration of the labelling solution throughout the mesothelium because in control embryos (reincubated for 3 h) only the mesothelial surface of the liver remained labelled. On the other hand, experiments *in vitro* have shown that this substance was unable to cross an epithelial barrier (Sun *et al.,* 2000).

All the results, taken together, suggest that mesenchymal cells delaminate from the surface mesothelium of the liver and some of them differentiate into endothelial cells which line the sinusoidal spaces between the hepatocytes. The rapidity of this process can

***Address correspondence to:** Ramón Muñoz-Chápuli. Departamento de Biología Animal, Facultad de Ciencias, Universidad de Málaga, Spain.
e-mail: chapuli@uma.es

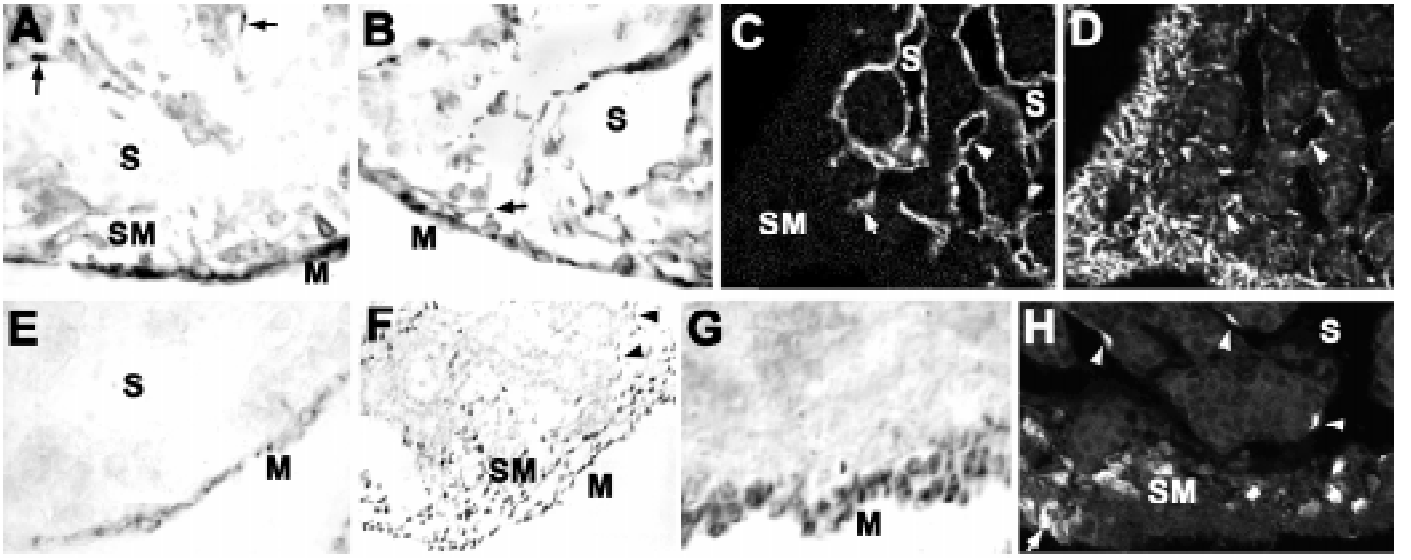

Fig. 1. Evidence of a contribution of mesothelial-derived cells to the sinusoidal endothelium of the embryonic avian liver. (A) *Chick, HH25. Cytokeratin (CK) immunoreactivity in the liver mesothelium (M), submesothelial mesenchyme (SM) and some cells (arrows) lining the sinusoids (S).* **(B)** *Chick, HH27. Vimentin immunoreactivity is also detected in the mesothelium, mesenchyme and sinusoidal endothelium. Note the cells apparently delaminating from the surface (arrow).* **(C,D)** *Quail, HH22. Colocalization, in the same confocal plane, of the endothelial marker QH1* **(C)** *and CK* ***(D)****. Note the colocalization in the sinusoidal walls (arrowhead) and also in cells located at the marginal areas, where new sinusoids are forming (arrow).* **(E)** *CK immunoreactivity has disappeared from the sinusoids and submesothelial mesenchyme by HH30.* **(F, G)** *Immunolocalization of the transcription factors Slug* ***(F)*** *and WT-1* ***(G)*** *in the mesothelium and mesenchyme of the quail liver (HH22 and 26, respectively). Isolated cells of the sinusoidal walls are Slug+ (arrowheads in F). H: When the liver surface is labelled with the fluorochrome CCFSE, the label is found, 24 h later, in the submesothelial mesenchyme but also in the mesothelium (arrow), and some sinusoidal cells (arrowheads). Note the lack of label in the hepatocytes.*

account for the transient retention of CK immunoreactivity in the sinusoidal cells, probably due to remains of the mesothelial intermediate filaments which are being replaced by vimentin. We had suggested that this model of *in situ* differentiation of angioblasts from the surface mesothelium also occurs in the developing heart (Pérez-Pomares *et al.,* 1997, 1998). The evidence of a similar process in an endodermal organ, such as the liver, provides support to our hypothesis about the embryonic coelomic mesothelium as a source of pluripotential cells. According to our hypothesis, the mesothelium would be induced to generate mesenchyme in those locations where the primordia of different organs are developing.

This study was supported by grant PM98-0219 and 1FD97-0693 from the Ministry of Education and Culture, Spain.

References

MOORE, A.W., MCINNES, L., KREIDBERG, J., HASTIE, N.D. and SCHEDL, A. (1999) YAC complementation shows a requirement for Wt1 in the development of epicardium, adrenal gland and throughout nephrogenesis. *Development* 126:1845-1857

MUÑOZ-CHÁPULI, R., PÉREZ-POMARES, J.M, MACÍAS, D., GARCÍA-GARRIDO, L., CARMONA, R. and GONZÁLEZ, M. (1999) Differentiation of hemangioblasts from embryonic mesothelial cells? A model on the origin of the vertebrate cardiovascular system. *Differentiation* 64:133-141.

NIETO, M.A., SARGENT, M.G., WILKINSON, D.G. and COOKE, J. (1994) Control of cell behavior by slug, a zinc finger gene. *Science* 264:835-839.

PÉREZ-POMARES, J.M., MACÍAS, D., GARCÍA-GARRIDO, L. and MUÑOZ-CHÁPULI, R. (1997) Contribution of the primitive epicardium to the subepicardial mesenchyme in hamster and chick embryos. *Dev. Dyn.* 210:96-105.

PÉREZ-POMARES, J.M., MACÍAS, D., GARCÍA-GARRIDO, L. and MUÑOZ-CHÁPULI, R. (1998) The origin of the subepicardial mesenchyme in the avian embryo: an immunohistochemical and quail-chick chimera study. *Dev. Biol.* 200:57-68.

RISAU, W. and FLAMME, I. (1995) Vasculogenesis. *Ann. Rev. Cell Dev. Biol.* 11:73-91.

SHERER, G.K. (1991) Vasculogenic mechanisms and epithelio-mesenchymal specificity in endodermal organs. In *The Development of the Vascular System* (Ed. R.N. Feinberg, G.K. Sherer and R. Auerbach), pp. 37-57. Karger, Basel.

SUN, D., GRIFFITH, C.M. and HAY, E.D. (2000) Carboxyfluorescein as a marker at both light and electron microscope levels to follow cell lineage in the embryo. In Methods in Molecular Biology, vol 135: *Developmental Biology Protocols*, Vol. 1 (Ed. R.S. Twan and C.W. Lo), pp. 357-363. Humana Press Inc., Totowa, NJ.