Research Article

Origin of pancreatic endocrine cells from biliary duct epithelium

D. Eberhard, D. Tosh and J. M. W. Slack

Abstract. We describe an explant culture system to study the formation of pancreatic-type endocrine cells by the biliary tract. In this model, β-cells and other endocrine cells appear in the biliary duct epithelium and their number increases. Evidence for an origin from the duct epithelium is threefold. Firstly, differentiating cells transiently co-express insulin and bind Dolichos lectin. Secondly, β-cells in cultures isolated from Alb-Cre-R26R-LacZ mice are β-galactosidase positive. Thirdly, co-culture of biliary epithelium and ROSA26 pancreatic buds shows that endocrine cells do not migrate from the pancreas. The expression of the pancreatic transcription factors Pdx1, HNF6 and Sox9 is widespread, as is Hes1, which represses endocrine development, while that of Ngn3, which is a proendocrine transcription factor, is transient, consistent with an early stage of endocrine cell differentiation. Nicotinamide will increase the number of β-cells formed, while EGF+LIF completely inhibits their formation.

Keywords. Pancreas development, liver development, β-cells, Pdx1, Hes1, Ngn3, extrahepatic biliary system, gall bladder development.

Introduction

The pancreas of higher vertebrates develops from a ventral and dorsal bud of the foregut endoderm [1–4]. These buds subsequently fuse and give rise to all pancreatic cell types including exocrine acini and ducts and endocrine islets of Langerhans containing five cell types secreting insulin (β-cells), glucagon (α-cells), somatostatin (β-cells), pancreatic polypeptide (PP-cells) or ghrelin (γ-cells) [5, 6]. Whereas cells secreting the last four hormones can also be found in the gut, and ectopic expression of insulin has been described in some pathological conditions [7], the development of β-cells was thought to be restricted to the pancreas alone. However, we have recently described another population of endocrine cells closely resembling or identical to those the pancreas, which is located in the extrahepatic biliary system of the mouse and arising in late embryonic development [8].

The extrahepatic biliary system consists of a set of extrahepatic bile ducts coming out of the liver, together with the gall bladder, the cystic duct connecting the gall bladder to the main duct system, and the common bile duct which joins the liver and pancreas to the small intestine. For clarity, we refer to this whole group of structures as the “extrahepatic biliary system”, while we reserve the term “extrahepatic bile ducts” just for those ducts that lie between...
the cystic duct and the liver (Fig. 1). Together with the liver and the ventral pancreas, the extrahepatic biliary system arises from a contiguous region of the ventral endoderm [9–11]. The insulin expressing cells were found as individuals or clusters in and along the biliary epithelium, but not in the gall bladder epithelium. They are likely to be genuine β-cells, as they process insulin (C-peptide positive), contain typical electron-dense insulin granules visible by electron microscopy, and are glucose responsive [8]. These findings suggest that the embryonic extrahepatic biliary system has the potential to produce β-cells. Indeed, the transcription factors Pdx1 (pancreatic and duodenal transcription factor 1, [12], HNF6 [13] and Hes1 (hairy enhancer of split) [14] which are expressed during pancreatic development are also expressed in the extrahepatic biliary system. Most strikingly, in mice lacking Hes1, the extrahepatic biliary epithelium converts to pancreatic tissue [14, 15], indicating that Hes1 represses pancreatic development in this region.

In the present study we examine the mechanism of production of endocrine cells by the extrahepatic biliary tract. For this purpose we have devised a new explant culture system of the liver hilar region, which is the region where major blood vessels enter and the bile ducts exit the liver. The cultures form a small amount of liver parenchyma, the main extrahepatic bile ducts, the gall bladder, the cystic duct and part of the common bile duct. We have identified individual cells in the duct epithelium which express pro-pancreatic transcription factors and are presumably the precursors for the endocrine cells. These cells are not found in the gall bladder. Two different types of cell labelling experiment show that the endocrine cells arise from the ducts in situ and do not migrate from the ventral pancreas. Additionally, we have studied the effect of exogenous factors and identified some that increase and some that decrease the number of β-cells arising from the ducts.

Materials and Methods

Isolation of embryonic extrahepatic biliary system.

Animal husbandry and embryo isolation were carried out in accordance with UK Home Office regulations. E15.5 stage mouse embryos were isolated from CD1 females which were sacrificed by cervical dislocation. Embryos were removed from the uterus and transferred to cold Minimum Essential Medium (MEM) with Hanks’ salts (Sigma), 10% fetal bovine serum (FBS, Invitrogen), 2 mM L-glutamine (Sigma), 1% penicillin/streptomycin (Sigma, P4333) and 20 μg/ml gentamicin (Invitrogen) and the liver with a part of the duodenum attached to the extrahepatic biliary system was dissected free. The components of the biliary system were then separated from liver tissue and adjacent tissues.

Biliary ducts were cultured on coverslips subbed with 3-aminopropyltriethoxysilane (APTES, Sigma) then coated with bovine plasma fibronectin (50 μg/ml, Invitrogen). The medium was DMEM (Gibco, 41966, 4.5 g/l glucose), 20% FBS, 1% penicillin/streptomycin (Sigma, P4333) and 20 μg/ml gentamicin. A cloning ring was placed over the fibronectin-coated area during the first two days to support attachment of the explant on the substrate. The medium was changed every two days. The cultures were grown at 37°C, 95% air/5% CO₂ in a humidified incubator for up to eight days.

Soluble factors/growth factors. To test the potential of various exogenous factors on the development of insulin-positive cells, per experiment, 6–8 ducts were randomly chosen on the day of isolation and cultured in high glucose DMEM medium containing a soluble factor (for concentrations see list below). Simultaneously, an equal number of ducts were grown in medium without factors as a control group. Inhibitors were added the following day, as we found they inhibit attachment of the ducts to the fibronectin matrix. The media with the factors was changed every day. After six days in culture, the average number of insulin-positive cells in treated and untreated ducts was determined. Each experiment was repeated three to five times and the average increase and S. E. M. was calculated and plotted.