Intestinal metaplasia in liver of rats after partial hepatectomy and treatment with acetylaminofluorene

V. Barut and C. E. Sarraf

Department of Biomedical Sciences, School of Biosciences, University of Westminster, London, UK

Received 16 January 2009; revision accepted 7 April 2009

Abstract

Objectives: The liver is widely recognized for its ability to self-regenerate after damage. Hepatocyte replication is the primary source of liver restoration, although hepatic stem cells (of one kind or another) may be a secondary font, only brought into effect when primary regeneration is severely compromised.

Materials and methods: In experiments using small rodents, such an injury can be inflicted by surgically removing a large portion of the liver followed by treatment with hepatotoxin 2-acetylaminofluorene. Regeneration by hepatocyte replication is blocked and thus, stem cell involvement is promoted. However, other responses may be stimulated and this study describes the presence of mucinous glandular structures in the healing liver after two-thirds of its volume was removed *via* hepatectomy followed by treatment with 2-acetylaminofluorene.

Results: Unique observation of intestinal metaplastic cells was seen under alcian blue/periodic acid–Schiff staining.

Conclusion: The existence of this phenotype (along with oval cells and small hepatocyte-like cells) is evidence of multipotency of progenitors involved in the hepatic healing response.

Introduction

The aim of this study was to document the presence and importance of intestinal metaplastic cells in rat livers after partial (two-thirds) hepatectomy followed by treatment with the hepatotoxin 2-acetylaminofluorene.

Under normal circumstances, non-lethal trauma to liver would be repaired by proliferation of hepatocytes. However, a large body of stem cell investigations has aimed to reveal that other cell types (bile duct cuboidal cells, oval cells, small hepatocyte-like cells, cells of the canals of Hering, bone marrow-derived stem cells (1–5 and reviewed by Alison *et al.* 6)) could fulfil this role of hepatocytes if the latter had become incapacitated. When hepatocyte proliferation has been experimentally impaired, biliary-like epithelial cell precursors have been seen to relocate towards and into the hepatic parenchyma while still expressing biliary cytoplasmic cytokeratins, until they develop expression of hepatocyte-specific alphafoetoprotein and albumin (7–9).

Liver diseases are suffered from globally, and exploitation of the existence of liver stem/progenitor cell multipotency offers the possibility of cell therapeutic protocols and development of alternative cell-based treatment, to alleviate the shortage of donor organs for standard transplantation. Currently, patients with chronic liver diseases, often with concomitant cirrhosis, have to experience a daunting wait, or die. Local multipotency is an acknowledged property of liver precursor cells, their embryological source being from endodermal pre-commitment gastrointestinal stem cells, responsible for both the biliary tree and the liver. Using autologous cell transplantation would be ideal for treatment, as it would overcome ethical dilemmas surrounding implantation of embryonic stem cells, and biological control of multipotentiality of hepatocyte precursors would be ideal for developing such therapy.

Materials and Methods

Tissue used in this investigation was archival, from rats that had been subjected to the Solt-Farber protocol. Hepatocyte regeneration induced by partial hepatectomy had been suppressed by 2-acetylaminofluorene treatment. The paraffin wax-embedded blocks used here were part of a research project (performed by Golding, 10). The

Correspondence: V. Barut, Department of Cardiovascular Medicine NHLI, Imperial College London, 5th Floor North Wing, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK. Tel: +44 7858621278; Fax: +44 (0)2083835513; E-mail: vedat.barut08@imperial.ac.uk

Figure 1. Oval cell migration. Oval cells are present in the lumina of sinusoids (thick arrows) and between hepatocytes (thin arrow). This is in agreement with the postulate that they move away from bile duct locations through the hepatocyte parenchyma. S, sinusoid; E, endothelial lining; H, normal hepatocyte; BD, bile duct; PV, hepatic portal vein. Haematoxylin and eosin stained, $\times 100$ original magnification.

modified Solt-Farber model had been applied to male Fischer rats. This involved administering 2-acetylaminofluorene to rats by oral gavage 6 days before and 7 days after partial hepatectomy. This surgical procedure activates liver regeneration; however, administration of 2-acetylaminofluorene inhibits liver regeneration, which then activates oval cells to compensate for lost liver mass. Liver samples from the rats were taken 4, 9 and 14 days after 2-acetylaminofluorene administration and then partial hepatectomy; samples were fixed and then embedded.

Blocks from this archive were serial-sectioned ($3\ \mu\text{m}$), stained with haematoxylin and eosin (H&E) using a standard method, and examined using a light microscope. Further sections were stained using the alcian blue/periodic acid-Schiff (AB/PAS) protocol for identification of and differentiation between acid and neutral mucins. Sections were stained with 1% alcian blue dye in 3% acetic acid for 5 min and after washing were subjected to 1% periodic acid solution for 5 min. After further thorough washing, sections were stained with Schiff's reagent for 15 min, before washing in running tap water for 10 min; no contrasting nuclear stain was used. Results were observed and photographed using light microscopy at $\times 40$ and $\times 100$ magnification of the objective lens.

Results

H&E staining showed significant oval cell populations near portal tracts and their emanation from these, largely seemingly *via* sinusoids, through the parenchyma of

Figure 2. Intestinal metaplasia. Quasi-glands are present in the parenchyma, central mucins (M) stained bright blue after the alcian blue/periodic acid-Schiff (AB/PAS) technique; this reveals their acid nature. H, hepatocytes; I, intestine-type cells. Diameters of central lumina 30–57 μm , total diameter of features 94–108 μm . AB/PAS stained, $\times 100$ original magnification.

hepatocytes (Fig. 1). This is as previously reported in a vast number of similar investigations. Serial sections stained with AB/PAS were seen to have a milieu of greyish, unstained, non-counterstained cells (hepatocytes/oval cells), among which startlingly bright blue areas were seen in mucinous glandular structures (Fig. 2). Such a result from staining with AB/PAS is typical for the presence of non-sulphated acid mucins, such as those synthesized by epithelial cells of the gastrointestinal tract (PAS alone stains neutral mucins magenta). On closer examination these areas were seen to represent lumina surrounded by colonocyte-like mucin-producing cells. Each of these 'quasi-glands' had an outer diameter in the general order of around 100 μm and diameters of the lumina were ~ 40 –50 μm . These structures were common in the tissues. In livers of untreated animals, no oval cells, other metaplastic nor pathological changes were present. The locations of oval cells bore no relationship to those of the mucinous glandular structures.

Discussion

Using H&E staining, oval cells were clearly visible in experimental sections, in contrast to liver architecture from control animals. Their existence, plus their relevance as precursors in severely stressed regenerating liver, has been well documented for many years (e.g. 7,11). The archival blocks used in this investigation had been prepared, and intestinal metaplasia was first noted in this exact tissue by Golding (10). The AB/PAS technique can

distinguish between acid mucins and neutral mucins and, thus, can demonstrate the presence of more than one type of mucin in a specific specimen, if such should exist. Application of alcian blue first prevents acid mucins from reacting with PAS. No haematoxylin counterstain was used in this part of the investigation to prevent confusion between different blue-stained compartments (nuclei dark blue, acid mucin bright blue).

Presence of colonocyte-like cells producing acid mucins in the liver has been noted before, but its relevance to potential cell therapies has not previously been explored and the present study provides proof of principle of the breadth of liver regenerating phenotypes. Being able to produce and control specific cell phenotypes at will opens the vista of possible treatments to cure degenerative diseases, such as liver failure, and understanding these cellular and molecular changes will take some of the weight off embryonic or adult stem cell approaches that are beset by ethical and/or immunological rejection difficulties. However, to establish such cell-based therapies and be able to convert a commonly found tissue progenitor cell into a useful phenotype required, it is essential as a first step to be able to comprehend then activate the molecular bases for potential phenotype conversion. This will surely involve changes in application of master cell-specific transcription factors.

Intestinal metaplasia is defined as reversible modification of a fully differentiated cell type (in this case, hepatocyte or hepatocyte precursor) to another cell type (12) mucinous colonic-type. The metaplasia witnessed could be a pathway for liver cell differentiation, or could be a further downstream lineage into which oval cells develop (13) or *vice versa*; however, the results described here have no temporal component. It has been postulated that high dose 2-acetylaminofluorene slows down oval cell differentiation (promoting basophilic small hepatocyte growth) together with developing intestinal metaplasia and bile duct formation (14). Development of intestinal metaplasia in midzonal liver parenchyma (15) has been credited as having been a definitive result of oval cell differentiation, but our study provided no evidence of oval cell presence in close proximity or with any relationship to areas where intestinal metaplasia was found.

It is not difficult to contemplate generation of metaplastic intestinal cells in regeneration of severely injured liver; cells regenerating a tissue under such conditions might easily be subject to altered or limited gene expression for key transcription factors, thus subsequent phenotype change (16). In human embryology, liver and the biliary system are close relatives of the digestive tract, both of which differentiate from the foetal gut tube, from around the 3rd to 4th week of development post-fertilization. Cells of the early gut express (among others) Wnt signalling

proteins, and those of the liver (in animal studies at least) have been shown to express *Prt*, a Wnt2b homologue (17). In addition, hepatocyte nuclear factor 4 alpha regulates goblet cell maturation in the mouse colon (18). In a case of biliary papillomatosis, dilated bile ducts have been found containing mucinous material; papillary structures had mucin-producing columnar cells, reminiscent of gastrointestinal tract histology (19). Upregulation of CDX2 and MUC2 expression, *via* Toll-like receptors (20) and nuclear factor-kappaB signalling, have been related to development of intestinal metaplasia associated with bile ducts. The CDX2 protein, as described by Traber and Silberg (21), is the product of *CDX2*, a homeobox gene specific to intestinal cells (22), and MUC2 is a mucin protein found in the gastrointestinal system, secreted by goblet cells of the epithelial lining (23). There is also evidence of pancreatic metaplasia in differentiating hepatic tissue (24), and natural and spontaneous biliary insulin-producing cells have been observed on occasion in the mouse extra-hepatic biliary system, revealing the presence of an opportunity to control phenotype conversion, in this case to provide insulin-producing cells, as a cell-based therapy for diabetes. A recent study by Kuo *et al.* (25) documents pancreatic acinar cells being found in liver tissue explants. In patients with primary cholangitis, Lewis *et al.* (26) described such a wide variety of types of intermediate neoplasia (72 gallbladders from 100 consecutive liver explants) that they considered pyloric metaplasia and intestinal metaplasia to contribute evidence of a metaplasia-dysplasia-carcinoma sequence in these tissues.

As future work, to unequivocally identify the cells of our quasi-glands as of truly gastrointestinal lineage, immunocytochemistry (at least) should be performed to discover any expression of, for example, the CDX2 protein. Similarly, expression of guanylyl cyclase C could be sought, as this has been described in adenocarcinomas of intestinal metaplastic origin (27), or expression of trefoil factors. Trefoil factor-3 is expressed in normal intestinal epithelial cells, usually associated with the mucin-producing layer of the gastrointestinal tract (28) and also in developing malignancy throughout the adenoma-carcinoma sequence (29). It should also be noted that this is a preliminary report and discovery of intestinal metaplasia in this work forms a base for future studies. Further investigation could involve immunohistochemical staining of proteins, such as CDX2, MUC2, ITF, and RELM-beta, to elucidate the source of the intestinal metaplasia reported and to discover whether it is a derivative of oval cells.

Conclusion

Together with hepatocytes' ability to refurbish damaged liver under normal conditions, after partial hepatectomy

and 2-acetylaminofluorene treatment, it is now clear that hepatocyte-like and/or oval cells appear, with at least two further pathways of metaplasia (intestinal and pancreatic). This stresses multipotential features of precursor cells and their ability to differentiate down a variety of pathways in addition to hepatocytes and biliary cells. The evidence described above of independent intestinal metaplastic development in healing liver after severe damage indicates that such phenotypes arise when strict hepatic control of lineage succession is lost, and it is suggested that further study to harness such potential may help in development of cell-based therapies for chronic liver disease and liver failure.

References

- Alison M, Golding M, Lalani EN, Nagy P, Thorgerisson S, Sarraf C (1997) Wholesale hepatocytic differentiation in the rat from ductular oval cell, the progeny of the biliary stem cells. *J. Hepatol.* **26**, 343–352.
- Yasui O, Miura N, Terada K, Kawarada Y, Koyama K, Sugiyama T (1997) Isolation of oval cells from Long-Evans Cinnamon rats and their transformation into hepatocytes *in vivo* in the rat liver. *J. Hepatol.* **25**, 329–334.
- Alison M (1998) Liver stem cells: a two compartment system. *Curr. Opin. Cell Biol.* **10**, 710–715.
- Alison M, Sarraf CE (1998) Hepatic stem cells. *J. Hepatol.* **29**, 676–682.
- Alison MR (2003) Characterisation of the differentiation capacity of rat-derived hepatic stem cells. *Semin. Liver Dis.* **23**, 325–336.
- Alison MR, Vig P, Russo F, Bigger BW, Amofah E, Themis M, Forbes S (2004) Hepatic stem cells: from inside and outside the liver? *Cell Prolif.* **37**, 1–21.
- Golding M, Sarraf C, Lalani EN, Anilkumar TV, Edwards RJ, Nagy P, Thorgerisson SS, Alison MR (1995) Oval cell differentiation into hepatocytes in the acetylaminofluorene-treated regenerating rat liver. *Hepatology* **22**, 1243–1253.
- Golding M, Sarraf C, Lalani EN, Alison MR (1996) Reactive biliary epithelium: the product of a pluripotent stem cell compartment? *Hum. Pathol.* **27**, 872–884.
- Sarraf C, Lalani EN, Golding M, Anilkumar TV, Poulsom R, Alison M (1994) Cell behaviour in the acetylaminofluorene-treated regenerating rat liver. Light and electron microscopic observations. *Am. J. Pathol.* **145**, 1114–1126.
- Golding MC (1997) *The behaviour of liver epithelial cells during regeneration and carcinogenesis*. PhD Thesis, Department of Histopathology, Hammersmith Hospital, Imperial College London, UK.
- Alison MR, Poulsom R, Jeffery R, Anilkumar TV, Jagoe R, Sarraf CE (1993) Expression of hepatocyte growth factor mRNA during oval cell activation in the rat liver. *J. Pathol.* **171**, 291–299.
- Gutiérrez-González L, Wright NA (2008) Biology of intestinal metaplasia in 2008: more than a simple phenotypic alteration. *Dig. Liver Dis.* **7**, 510–522.
- Lowes KN, Croager EJ, Olynyk JK, Abraham JL, Yeoh CT (2003) Oval cell-mediated liver regeneration: role of cytokines and growth factors. *J. Gastroenterol. Hepatol.* **18**, 4–12.
- Evarts PR, Nagy P, Nakatsukasa H, Marsden E, Thorgerisson SS (1989) *In vivo* differentiation of rat liver oval cells into hepatocytes. *Cancer Res.* **49**, 1541–1547.
- Tatematsu M, Kaku T, Medline A, Farber E (1985) Intestinal metaplasia as a common option of oval cells in relation to cholangiofibrosis in liver of rats exposed to 2-acetylaminofluorene. *Lab. Invest.* **52**, 354–362.
- Eberhard D, Tosh D (2008) Transdifferentiation and metaplasia as a paradigm for understanding development and disease. *Cell Mol. Life Sci.* **65**, 33–40.
- Ober EA, Verkade H, Field HA, Stainier DY (2006) Mesodermal Wnt2b signalling positively regulates liver specification. *Nature* **442**, 688–691.
- Garrison WD, Battle MA, Yang C, Kaestner KH, Sladek FM, Duncan SA (2006) Hepatocyte nuclear factor 4 α is essential for embryonic development of the mouse colon. *Gastroenterology* **130**, 1207–1220.
- Güllüoğlu MG, Özden I, Poyanli A, Cevikbas U, Arioglu O (2007) Intraductal growth-type mucin-producing peripheral cholangiocarcinoma associated with biliary papillomatosis. *Ann. Diagn. Pathol.* **11**, 34–38.
- Ikeda H, Sasaki M, Ishikawa A, Sato Y, Harada K, Zen Y, Kazumori H, Nakamura Y (2007) Interaction of Toll-like receptors with bacterial components induces expression of CDX2 and MUC2 in rat biliary epithelium *in vivo* and in culture. *Lab. Invest.* **87**, 559–571.
- Traber PG, Silberg DG (1996) Intestine-specific gene transcription. *Annu. Rev. Physiol.* **58**, 275–297.
- James R, Kazenwadel J (1991) Homeobox gene expression in the intestinal epithelium of adult mice. *J. Biol. Chem.* **266**, 3246–3251.
- Gendler SJ, Spicer AP (1995) Epithelial mucin genes. *Annu. Rev. Physiol.* **57**, 607–634.
- Wolf HK, Burchette JL Jr, Garcia JA, Michalopoulos G. (1990) Exocrine pancreatic tissue in human liver: a metaplastic process? *Am. J. Surg. Pathol.* **14**, 590–595.
- Kuo FY, Swanson PE, Yeh MM (2008) Pancreatic acinar tissue in liver explants: a morphologic and immunohistochemical study. *Am. J. Surg. Pathol.* **33**, 66–71.
- Lewis JT, Talwalkar JA, Rosen CB, Smyrk TC, Abraham SC (2007) Prevalence and risk factors for gallbladder neoplasia in patients with primary sclerosing cholangitis: evidence for a metaplasia-dysplasia-carcinoma sequence. *Am. J. Surg. Pathol.* **31**, 907–1013.
- Birbe R, Palazzo JP, Walters R, Weinberg D, Schulz S, Waldman MD (2005) Guanylyl cyclase C is a marker of intestinal metaplasia, dysplasia, and adenocarcinoma of the gastrointestinal tract. *Hum. Pathol.* **36**, 170–179.
- Thim L (1997) Trefoil peptides: from structure to function. *Cell. Mol. Life Sci.* **53**, 888–903.
- Babyatsky M, Lin J, Yio X, Chen A, Zhang JY, Zheng Y, Twyman C, Bao X, Schwartz M, Thung S, Lawrence Werther J, Itzkowitz S (2008) Trefoil factor-3 expression in human colon cancer liver metastasis. *Clin. Exp. Metastasis* **26**, 143–151.