Vol. 156, No. 1, 1988
October 14, 1988

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 131-136

ALMOST TOTAL CONVERSION OF PANCREAS TO LIVER IN THE ADULT RAT: A RELIABLE MODEL TO STUDY TRANSDIFFERENTIATION

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Received August 25, 1988

Study of transdifferentiation provides an excellent opportunity to investigate various factors and mechanisms involved in repression of activated genes and derepression of inactivated genes. Here we describe a highly reproducible in vivo model, in which hepatocytes are induced in the pancreas of adult rats that were maintained on copper-deficient diet containing a relatively non-toxic copper-chelating agent, triethylenetetramine tetrahydrochloride (0.6% w/w) for 7-9 weeks and then returned to normal rat chow. This dietary manipulation resulted in almost complete loss of pancreatic acinar cells at the end of copper-depletion regimen, and in the development of multiple foci of hepatocytes during recovery phase. In some animals, liver cells occupied more than 60% of pancreatic volume within 6-8 weeks of recovery. Northern blot analysis of total RNA obtained from the pancreas of these rats revealed the expression of albumin mRNA. Albumin was demonstrated in these pancreatic hepatocytes by immunofluorescence. The advantages of this model over the previously described models are: a) low mortality (10%), b) depletion of acinar cells, and c) development of multiple foci of hepatocytes in 100% of rats.

Transdifferentiation is an irreversible process in which one differentiated cell is converted into a completely different cell type (1). During this process, they completely lose the morphological and biochemical characteristics of the original cell and acquire features of an entirely different cell type accompanied by expression of new genes (2). This fascinating phenomenon of transdifferentiation has been clearly documented in in vitro and in vivo conditions, shown to be possible with or without DNA synthesis and cell replication and can occur between cell types belonging to the same cell class, same cell lineage, or different cell lineages (2-5). Although transdifferentiation has been observed in several cell types, the histogenesis still remains controversial. It is not clear whether transdifferentiation involves direct conversion of a fully differentiated cell, conversion of an intermediate cell, or dedifferentiation of a fully differentiated cell followed by

0006-291X/88 $1.50

redifferentiation. To satisfactorily answer these questions, a reproducible experimental system which does not involve embryonic tissue is required.

Recently, we and others have described the development of hepatocytes in the pancreas of adult hamsters and rats that were either given carcinogens or maintained on a copper-depletion-repletion protocol, or a methionine-deficient diet (6-8). In these species, the induced pancreatic hepatocytes were identical to the liver parenchymal cells both morphologically and functionally (9,10). Although, the exact cell or cells from which pancreatic hepatocytes are derived in rats and hamsters is not established, the process of conversion is referred to as transdifferentiation, since hepatocytes are developing in an organ of an adult animal in which no stem cells are known to exist. The existing rat models are useful to study transdifferentiation and identify various factors that can lead to the development of pancreatic hepatocytes. However, these models are not ideal because of a high incidence of mortality, very low incidence of pancreatic hepatocytes, and the presence of almost all pancreatic acinar cells. In the present study, we describe a highly reproducible model in which hepatocytes are induced in the pancreas of rats that are maintained on a copper-deficient diet supplemented with 0.6% triethylenetetramine tetrahydrochloride (trien) for 50-65 days and returned to normal diet. The advantage of this model is less than 10% mortality of animals and extensive pancreatic hepatization in 100% of the animals in a pancreas which has been depleted of most of its exocrine acinar cells.

Materials and Methods

Twenty male Fischer 344 rats, weighing 80-90 g, were purchased from Charles River Breeding Laboratories (Wilmington, MA). They were housed in individual plastic cages with stainless steel wire bottoms. All rats were fed a copper-deficient diet (United States Biochemical Corp., Cleveland, OH) supplemented with 0.6% triethylenetetramine tetrahydrochloride (trien) (Aldrich Chem.Co.) for 7-9 weeks. During the final two weeks, if the animals were found sick they were returned to a normal diet (Purina rat chow, St.Louis, MO); otherwise, all rats were changed to a normal diet on the 65th day. Ten control rats were maintained on a normal diet throughout the experimental period. Five animals from experimental and five from control group were sacrificed at the end of copper-deficiency regimen. Eight weeks after changing to a normal diet, all rats were sacrificed. The pancreas from all animals was processed for light and electron microscopy. For immunofluorescence localization of albumin, portions of pancreas were fixed in 70% ethanol and processed as described (7). Total RNA was isolated from liver, normal pancreas and pancreas with hepatocytes after homogenization in guanidinium isothiocyanate and centrifugation through cesium chloride (11), and analyzed by Northern blot hybridization using nick translated ^{32}P-labeled albumin cDNA (12).

Results and Discussion

Rats maintained on a copper-deficient diet containing 0.6% trien gained body weight during the first 6 weeks, although at a lower rate than control rats. At 8 weeks of copper deficiency, experimental animals weighed 20% less than controls. The pancreas

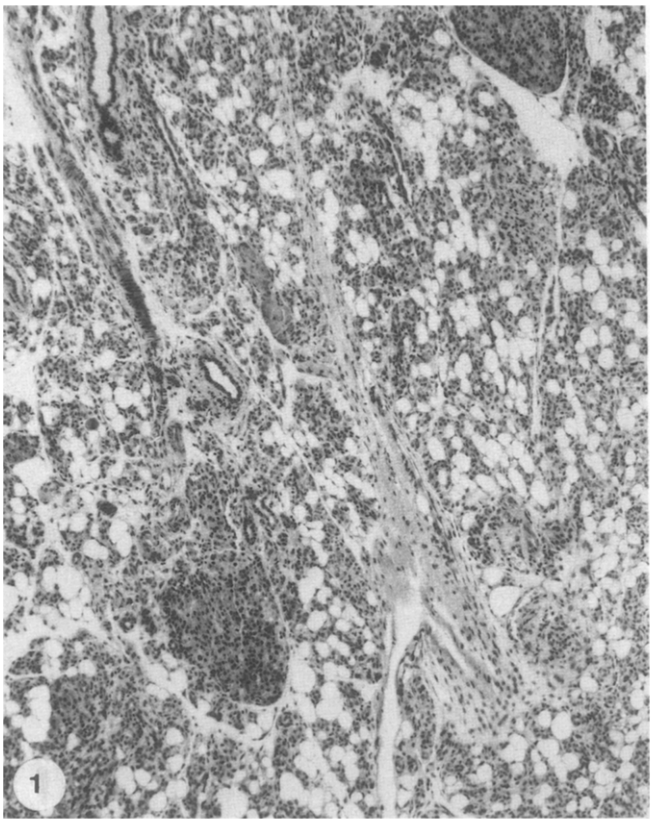

Fig.1 Pancreas of a rat maintained on a copper-deficient diet containing 0.6% trien for 8 weeks, shows total loss of acinar tissue, collapse and fatty infiltration. H&E, X120.

of all of 5 rats that were killed at the end of 8 weeks of copper-deficiency regimen was markedly atrophic and weighed approximately 90 mg/100 g body weight as compared to about 230 mg/100 g body weight in controls. Histologically, there was extensive (>90%) loss of acinar tissue (Fig.1). Islets and ducts appeared unaffected. The interstitial tissue contained increased numbers of spindle or oval-shaped cells.

The pancreas of all 13 rats that were killed 8 weeks after changing to a normal diet showed fatty infiltration along with multiple foci of randomly distributed hepatocytes. Although, there was some variation in the number of hepatic foci between animals, in a majority of rats more than 60% of the pancreas was occupied by hepatocytes (Fig.2). Size of the hepatic foci ranged from groups of few cells to large clusters of several cells. Morphology of pancreatic hepatocytes was indistinguishable from liver parenchymal cells. These cells are polyhedral with a central nucleus containing a prominent nucleolus and substantial eosinophilic cytoplasm. Electron microscopic appearance of pancreatic hepatocytes was indistinguishable from that of liver hepatocytes. Some of the liver cells were seen in continuity with smaller intercalated ducts, or in close association with islets, whereas others were present in the fatty stroma

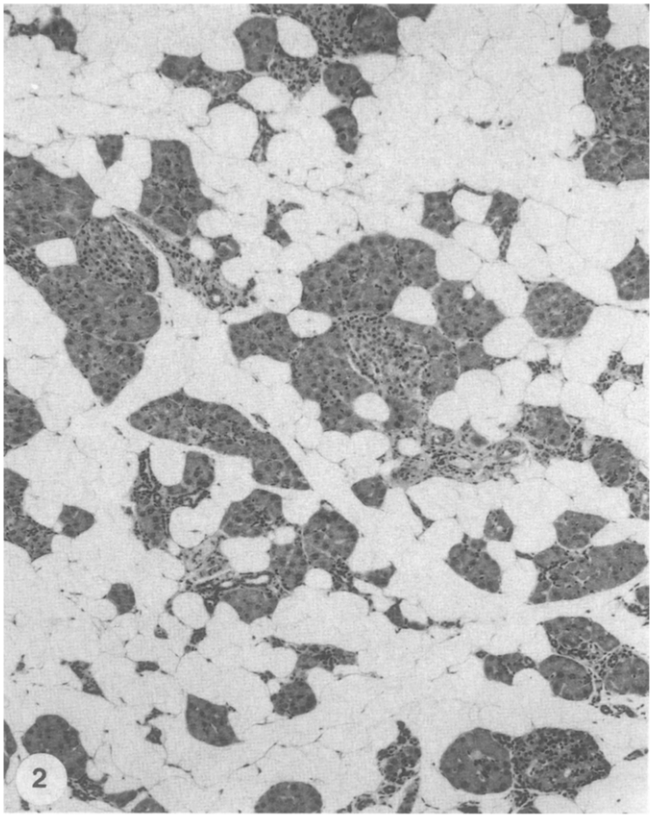

Fig.2 Pancreas of a rat maintained on a copper-deficient diet containing 0.6% trien for 8 weeks and changed to normal diet for 8 more weeks, showing multiple foci of randomly distributed hepatocytes. H&E, X120.

without any apparent relation to either ducts, acini or islets. Detailed analysis of 0.5μm thick sections with light microscopy suggested that hepatocytes appear to originate from epithelium of small ductules (intercalated ducts) and scattered interstitial cells. The amount of acinar tissue in the pancreas of these animals was less than 15% of total volume. Total RNAs were isolated from the pancreas of two rats killed at 8 weeks of recovery from copper-deficiency and analyzed by Northern blot method to ascertain the expression of mRNA for albumin, a liver specific protein. For comparison, total RNA isolated from normal rat liver and pancreas was also analyzed (Fig.3). Albumin mRNA is detected in the pancreas of both rats recovering from copper-deficiency (Fig.3, Lanes 1&2) but not detected in normal rat pancreas (Fig.3, Lane 3). Furthermore, albumin was localized in all hepatocytes in these pancreata by indirect immunofluorescence method (not illustrated).

With this dietary regimen, coupled with a less toxic copper-chelator, trien, only 2 rats (10%) died during the entire experimental procedure, as compared to 50% mortality observed in rats that were made copper-deficient using D-penicillamine as chelator (7). Although penicillamine and trien are equally effective in inducing cupruresis, the

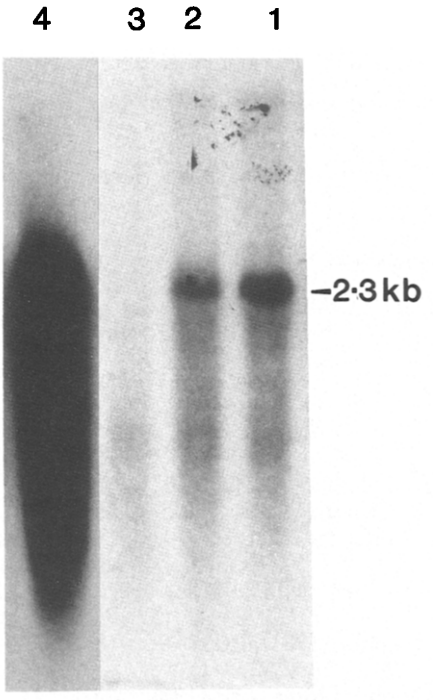

Fig.3 Northern blot analysis of the albumin mRNA in the pancreas of two rats 8 weeks after recovery from copper-depletion-induced atrophy (Lanes 1&2). Lanes 3&4 represent RNA isolated from pancreas and liver respectively from a normal rat. Total RNA (15 μg/Lane) was denatured with glyoxal, electrophoresed, transferred to nylon filter, and hybridized with a ^{32}P-labeled albumin cDNA. The autoradiograph demonstrates a 2·3 kb mRNA in liver and pancreas of two rats with hepatocytes, but not in normal rat pancreas.

increased mortality of rats in experiments using penicillamine is due to its toxic effects in several organs and multiple enzyme systems (13,14). In addition to low mortality, the number of foci and incidence of hepatocytes in the pancreas of rats maintained on copper-deficient diet with trien are high and reproducible. The marked depletion of acinar cells in these pancreata makes this system highly attractive for studying trans-differentiation

The mechanism(s) by which copper deficiency induces hepatic transdifferentiation in pancreas is not clear. The differentiated state of a cell is probably dependent on DNA methylation, chromatin structure, and DNA protein interactions (15,16). Copper is an important trace metal and is essential for the activity of several enzymes (17). Deprivation of cells from copper can lead to macromolecular changes which may result in expression of new genes. In addition to DNA alterations, cell-cell interactions may also play an important role in the maintenance of differentiated state (18). Since copper deficiency leads to almost total ablation of acinar tissue, this may result in loss of feedback mechanism, resulting in proliferation of ductular and interstitial cells with altered differentiation. The experimental model described here should be invaluable in

studying the role of cells lining the intercalated and smaller pancreatic ducts in the histogenesis of hepatocytes and in elucidating the molecular events involved in transdifferentiation of these pancreatic cells to hepatocytes.

Acknowledgment

This research was supported by Grant DK 37958 from The National Institutes of Health.

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