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Transdifferentiation and metaplasia as a paradigm for understanding development and disease

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Abstract. The ability to produce differentiated cell types at will offers one approach to cell therapy and therefore the treatment and cure of degenerative diseases such as diabetes and liver failure. Until recently it was thought that differentiated cells could only be produced from embryonic or adult stem cells. However, we now know that this is not the case, and there is a growing body of evidence to show that one differentiated cell type can convert into a completely different phenotype (transdifferentiation). Understanding the cellular and molecular basis of transdifferentiation will allow us to reprogram cells for transplantation. This approach will complement the use of embryonic and adult stem cells in the treatment of degenerative disorders. In this review, we will focus on some well-documented examples of transdifferentiation. (Part of a Multi-author Review)

Keywords. Transdifferentiation, metaplasia, development, neoplasia, cancer, acinar-to-ductal metaplasia, master switch genes.

Introduction

In general, the conversion of one cellular or tissue phenotype to another is termed metaplasia and can include conversions between stem cells as well as direct conversion of differentiated cells (Fig. 1) [1, 2]. Transdifferentiation is a subclass of metaplasia and by definition an irreversible switch of one already differentiated cell to another, resulting in the loss of one phenotype and the gain of another [3]. There is a third cell-type switch termed heterotopia, which refers to a switch in phenotype of a cell during embryogenesis. Metaplasia and transdifferentiation are nearly always found in association with tissue damage and regeneration. Presumably this occurs because regeneration facilitates changes in the expression of key transcription factors, which in turn leads to an alteration in the developmental commitment of a cell. These changes can be brought about by somatic mutation of environmental changes. One well-defined example is provided by lens regeneration [4]. This will be dealt with in more detail elsewhere (see review by Tsonis in this issue).

There are two important experimental criteria that need to be established for a process to be considered as a transdifferentiation. First, the phenotype of the cell before and after transdifferentiation should be clearly defined using morphological appearance and biochemical and/or molecular evidence. Defining the phenotype could be achieved through large-scale genetic or proteomic screening of the cells as well as by functional characterization. Second, the cell lineage (ancestor-descendant) relationship between the two cell types needs to be clearly established [3]. The ancestor-descendant analysis nearly always requires some form of lineage labelling, although sometimes it can be established by direct observation of the cells in tissue culture. Cell division often accompanies transdifferentiation, but there are cases where it does not do so [5].

Studying transdifferentiation is important for a number of reasons. The first is because harnessing transdifferentiation and metaplasia will allow us to develop

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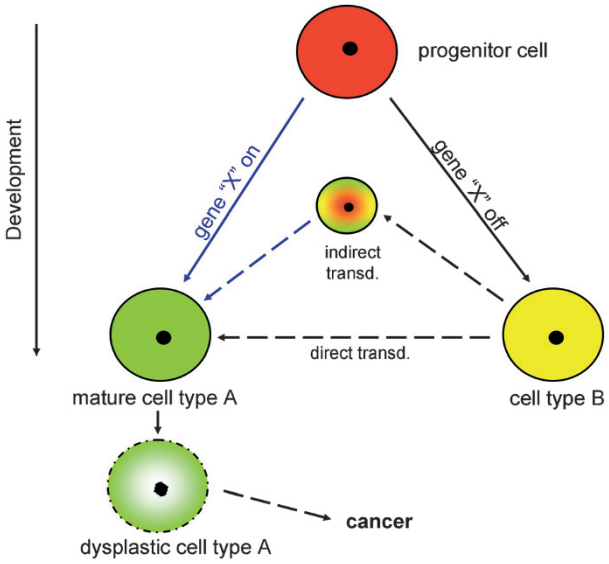

Figure 1. Relationship between transdifferentiation, development (differentiation) and disease. *Development:* Differential gene activity of a master switch 'gene X' in progenitor cells (with marker shown in red) leads to the acquisition of the properties of mature functional cells during development (differentiation) of two cell types 'A' and 'B'. These cells can be distinguished by the differentiation markers (green and yellow). Transdifferentiation (dashed arrows): The change of one differentiated cell type (B) to another (A) by direct transdifferentiation (lower dashed arrow) or indirectly featuring transitional cells expressing a mixture of markers found in cell types A and B (green and yellow). Transitional cells might express markers/transcription factors (red) found in the progenitors of both cell types (red) and reactivation of gene 'X' may mediate redifferentiation. Metaplasia (the conversion of one cell to another) includes the conversion from tissue-specific stem cells, i.e. the conversion from cell type B to A, if B was a stem cell. Metaplasia can lead to cells which are abnormal (dotted arrow) and may predispose to cancer.

alternatives for therapeutic transplantation for treating and curing degenerative diseases. Targets for such diseases include diabetes and liver failure. We will focus on examples of transdifferentiation of pancreas and liver later in the review. The second reason for studying transdifferentiation and metaplasia is because changes in the cellular phenotype can predispose to the development of cancer. Perhaps one of the best-known examples of this type of switch in cellular phenotype is Barrett's metaplasia, whereby the lower end of the oesophagus changes from stratified squamous epithelium to intestinal-type columnar epithelium [6]. Barrett's metaplasia predisposes to oesophageal adenocarcinoma [7]. Identification of the key regulatory mechanisms underlying the switch from oesophageal-type tissue to intestinal-type tissue is essential for the early diagnosis and treatment of the diseases. During the review we will also look at the molecular basis of Barrett's metaplasia. The third reason for studying transdifferentiation and metaplasia is related to the identification of the transcription factor(s) that are responsible for the switch in phenotype. The identification of transcription factors involved in transdifferentiation and metaplasia is important for two reasons. First, the gene(s) that induces transdifferentiation tells us something about the normal developmental biology of the two tissues that interconvert. During embryogenesis, tissues that develop from neighbouring regions in a common cell sheet will have similar combinations of transcription factors defining their commitment. Neighbouring tissues may therefore differ by the expression of just one or two transcription factors. At the molecular level, the cause of transdifferentiation is presumably a change in the expression of a master switch gene (homeotic or selector gene), whose normal function is to distinguish the cell types in normal development. The second reason is that understanding the molecular rules for cell or tissue-type conversions will improve our ability to reprogram stem cells for the purpose of therapeutic transplantation.

The role of Pdx1 in pancreatic acinar-to-ductal metaplasia

Acinar-to ductal metaplasia is an example of the conversion between two cell types within the same tissue, the pancreas. The normal adult pancreas is composed mainly of exocrine acinar tissue with ductal and endocrine cells scattered throughout [8]. Metaplastic changes replace acinar tissue with cells of a ductal phenotype. The switch from acinar to ductal cell types predisposes to neoplasia (e.g. pancreatic ductal adenocarcinoma, PDAC). PDAC is by far the most abundant pancreatic cancer [9]. PDAC cancer cells share many features of normal ductal cells, but the origin of the neoplastic cells has been the subject of debate as they could originate either by overgrowth of pre-existing ducts, from tissue-specific stem cells or by conversion from exocrine cells [9]. Recently, the cell origin as well as the underlying molecular mechanisms of acinar-to-ductal metaplasia have been addressed experimentally. Conversion of an acinar to a ductal phenotype can be induced in animal models by chemical or physical damage (pancreatic duct ligation or partial pancreatectomy) [10]. Acinar-to-ductal metaplasia could be observed *in situ* in transgenic mice following overexpression of transforming growth factor α (TGFα, [11], interferon γ (IFNγ [12] and the pancreatic duodenum homeobox gene 1 (*Pdx1* [13] or *in vitro* after treatment with TGFα [14]), and even spontaneous acinar-to-ductal conversion has been reported [15].

Miyatsuka and co-workers have modelled acinar-to-ductal metaplasia by constitutive overexpression of

Table 1. Examples of metaplasia: summary of different types of metaplasia, including the molecular basis of the switch in phenotype where known.

Metaplasia context	Cell type switch	Molecular control	Comments	Refs.
Oesophagus: Barrett's metaplasia – pathological – experimental (in vivo and in vitro)	Lower oesophagus squamous epithelium to intestinal-type epithelium	Cdx2 bile acids RA	Predispose to oesophageal carcinoma	[6, 7] [45–52]
Pancreas: acinar to ductal metaplasia – spontaneous in vitro – experimental (in vivo and in vitro)	Exocrine acinar cells to ductal cells	TGFα, IFNγ Pdx1, Jak/Stat pathway EGF receptor	Predispose to neoplasia (PDAC: pancreatic ductal adenocarcinoma)	[9–16]
Liver to pancreas metaplasia – experimental in vivo	Hepatocytes to pancreatic β-cells	Pdx1 Pdx1 + Ngn3 Pdx1 + NeuroD	Cell reprogramming as a route to curing diabetes	[21–24]
Pancreatic heterotopia – spontaneous	Presence of insulin-expressing cells in non-pancreatic endodermal tissues (extra-hepatic bile ducts)	Hes1 Ngn3 pro-endocrine pancreatic TF		[28–31]
Pancreas to liver – pathological (cancer) – experimental (in vivo and in vitro)	Pancreatic cells (origin?) to heptocytes (foci in pancreas)	Copper deficiency KGF IFNγ C/EBP		[32–40]
Liver to bile duct – pathological (liver damage, chronic liver disease)	Hepatocytes to intrahepatic biliary	insulin, EGF EGF pathway CK19 duct cells		[41–44]
Uterus metaplasia – spontaneous upon DES	Uterine and cervix squamous epithelia to stratified epithelium	DES Msx2	Predispose to neoplasia: vaginal adenocarcinoma	[53–56]

Pdx1 transcription factor. *Pdx1* is thought to be a master switch gene for the formation of the pancreas, as homozygous *Pdx1* knockout mice lack a pancreas [16]. Pdx1 is normally expressed in the entire epithelial bud, but becomes progressively restricted to endocrine β-cells [8]. Miyatsuka et al. altered the pattern of Pdx1 expression in acinar cells or all pancreatic cells in transgenic mice using a Cre/Lox approach (Fig. 2). In these transgenic mice, cells constitutively express Pdx1 under control of a β-actin promoter after Cre recombinase-mediated removal of a stop sequence between the promoter and the Pdx1 coding region. Cre itself was either driven by the endogenous promoter of the pancreatic transcription factor Ptf1a or by an amylase promoter. As Ptf1a is expressed in all pancreatic lineages during development [17], Pdx1 was expressed in exo- and endocrine cells. In case of the amylase-Cre transgenic line, Pdx1 expression was restricted to more mature acinar cells.

The overexpression of Pdx1 in any of the mouse lines described above lead to the replacement of carboxypepidase A-positive acinar tissue by cells positive for duct-specific cytokeratins. As the acinar-to-ductal metaplasia appeared exclusively after birth, the conversion does not result from ectopic Pdx1 expression in pre-differentiated pancreas. Thus, overexpression of Pdx1 is able to induce acinar-to-ductal metaplasia in acinar cells. This acinar-to-ductal conversion is probably mediated by the JAK/Stat pathway, as increased levels of activated Stat3 (signal transducer and activator of transcription 3) were observed after Pdx1 overexpression. Strikingly, acinar-to-ductal transdifferentiation was restored in Stat3-deficient mice overexpressing Pdx1 in all pancreatic lineages [13].

This discovery is important because in human patients (and animal models) with pancreatic cancer or pancreatitis, upregulation of Pdx1 may be activating the JAK/Stat3 pathway, causing acinar-to-ductal metaplasia. Pharmacologic inhibition of the JAK/Stat3 pathway may provide a therapeutic strategy to suppress initiation of meta- and neoplasia.

In vitro culture experiments revealed an alternative pathway mediating acinar-to-ductal metaplasia by signalling through the EGF (epidermal growth factor) receptor [14]. Treatment of primary explant cultures of acinar cells with TGFα-induced acinar-to-ductal

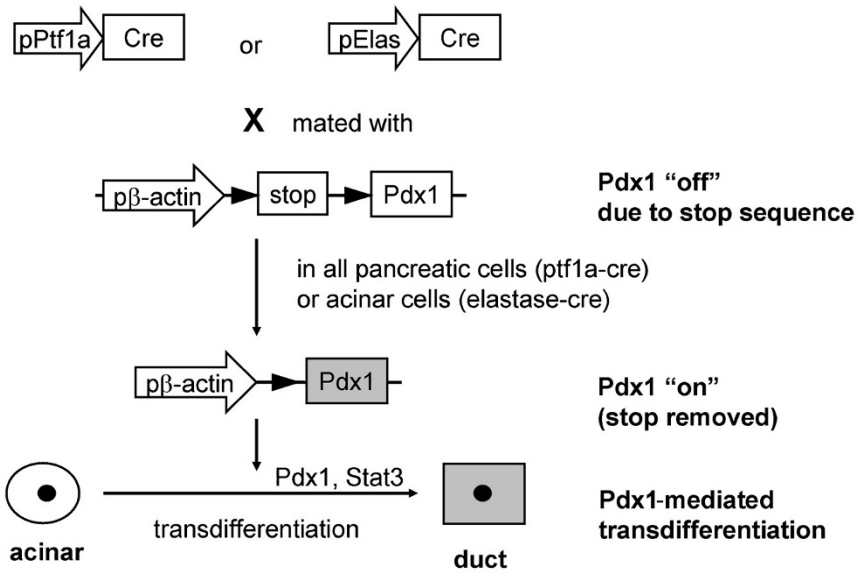

Figure 2. In the double transgenic mice used by Miyatsuka et al. [13], *Pdx1* is expressed under a β-actin promoter after a stop sequence flanked by loxP sites (black arrows) that has been removed by Cre recombinase. Cre itself is either driven by the Ptf1a (expressed in all pancreatic lineages during development) or the elastase gene (acinar cell-specific) promoters. Transdifferentiation is mediated by Pdx1 and Stat3. Alternatively, Pft1a::Cre β-actin Pdx1 mice were bred into Stat3 knockout mice in which the metaplastic phenotype was profoundly suppressed.

conversion and inhibition of TGFα-receptor signalling with the EGF receptor/erbB2 tyrosine kinase inhibitor EKI-785 prevents conversion. During acinar-to-ductal conversion, transient cells positive for the intermediate filament nestin were present, suggesting that acinar cells dedifferentiate before redifferentiating to duct cells [14]. Nestin has been proposed to be a marker of pre-differentiated pancreatic cells, but this view has been challenged recently, as only a subset of mature pancreatic cells arise from nestin-positive progenitors [18, 19].

Spontaneous acinar-to-ductal transdifferentiation can also be observed *in vitro* in cultures of acinar cells [15]. In this example, acinar cells started to express cytokeratin7 (CK7) and fetal liver kinase (Flk-1). Flk-1 is a vascular endothelial growth factor receptor and is normally expressed in adult pancreatic ducts [20]. Interestingly, the Pdx1 gene was reactivated in transdifferentiating cells, whereas Ptf1a (characteristic for mature acinar cells) continued to be expressed in transdifferentiating cells, suggesting a partial event.

Liver-to-pancreas metaplasia: hepatocytes to pancreatic β-cells

Diabetes mellitus constitutes a major health care problem. Approximately 180 million people worldwide suffer from diabetes [20a]. In type 1 'insulin-dependent' diabetes, the insulin-producing pancreatic β-cells are diminished or completely absent usually due to an autoimmune reaction directed against β-cells. Although insulin therapy for diabetes has been highly successful, the disease still produces a considerable burden of distressing complications. One possible route to curing diabetes is through production of β-cells by reprogramming other cell types via activation of genes normally involved in pancreas development [21]. Examples of cell types include hepatocytes, since they develop from the same germ layer (endoderm). Horb et al. used a hepatocyte-specific transthyretin (TTR) promoter that drives a superactive form of the Pdx1 homologue XlHbox8, XlHbox8VP16, in *Xenopus laevis* tadpoles to convert parts of the liver to pancreas [22]. Pdx1 alone [23, 24] or Pdx1VP16 in combination with other proendocrine pancreatic transcription factors such as Neurogenin (Ngn3) and NeuroD [25], have been delivered to mouse hepatocytes using adenoviral vectors, and transplanted cells were sufficient to alleviate hyperglycaemia induced by streptozotocin. A set of criteria are used to prove that β-cells are genuine [26]. These include expression of insulin RNA, the ability to process insulin from the precursor state, immunoreactive insulin secretory vesicles in β-cells, secretion of insulin in response to glucose and amelioration hyperglycaemia in diabetic mice [26]. The use of cells reprogrammed to insulin-producing β-cells could enable a cell-based diabetes therapy superseding insulin injections.

Insulin-expressing cells in the extrahepatic bile ducts

An example of heterotopia is provided by the biliary tract and the pancreas. The liver, biliary system and ventral pancreas arise from a common region of the ventral foregut endoderm. Experiments in mice and chicks have shown that the hierarchy of transcription

factors that encode for these tissues in development are very similar, and minor changes can alter their fate. For example, the liver endoderm is bipotential (can generate both hepatocytes and cholangiocytes) and expresses pancreatic markers in the absence of signals from the cardiac mesoderm [27]. Therefore, it is not surprising that pancreatic tissue can frequently be found in stomach, intestine or liver in various species, including humans [28]. Many of these cases are probably caused by developmental changes and are therefore examples of heterotopia rather than metaplasia or transdifferentiation, as they occur independent of a pathological condition.

We have recently described a population of naturally occurring pancreatic endocrine cells (including β-cells) in the extrahepatic biliary system of the mouse [29]. Acinar pancreatic tissue was not present. These ectopic β-cells arise from the liver (albumin) domain [29], and the first biliary derived β-cells appear around E17.5 in the mouse embryo in the biliary epithelial lining in the liver hilar region. Although single β-cells can grow into larger clusters, the total number of foci does not increase in postnatal life, suggesting that these cells are only generated during a limited period in embryonic life. An error in the transcription factor code such that duct cells express a transcription factor combination inducing pancreas development is the most likely reason for generation of these ectopic cells. In some cases of cell-type conversions, the change in expression of a single transcription factor can lead to a change in the cellular phenotype. For example, ectopic pancreatic tissue replaces the extrahepatic bile ducts in mice deficient for the bHLH transcription factor Hes1 (hairy and enhancer of split1) [30]. Hes1 is regulated by the Notch/delta pathway and is a repressor of the pro-endocrine pancreatic transcription factor Neurogenin 3 (Ngn3). Hes1 is highly expressed in the developing extrahepatic bile ducts. In Hes1 knockout mice, extrahepatic biliary ducts ectopically express Ngn3, which might account for the conversion of the complete biliary primordium to endocrine and exocrine pancreatic tissue. In normal bile duct development, rare spontaneous instances of altered transcription factor (e.g. lower levels of Hes1 resulting in de-inhibition of Ngn3 expression) could lead to generation of ectopic pancreatic endocrine cells as seen naturally in the extrahepatic biliary system of the mouse. The presence of biliary insulin-positive cells has highlighted the possibility of reprogramming biliary tissue to β-cells to develop a cell therapy to cure diabetes [31].

Conversion of pancreas to liver

Conversion of pancreatic cells to hepatocytes has been observed in various pathological and experimental situations. For example, in rats fed with a copper-deficient diet and the copper-chelating agent triethylenetetramine, more than 60% of the pancreas converts to albumin-positive hepatocytes a few weeks after returning to a normal diet [32, 33]. Transgenic mice ectopically expressing keratinocyte growth factor (KGF) [34] or IFNγ [12] under the control of an insulin promoter also lead to foci of hepatocytes in the pancreas. In human pathology, hepatic tissue has been observed in the pancreas in association with cancer [35]. Due to lack of appropriate lineage-tracing tools in all of these studies, the origin of the pancreatic hepatocytes is unknown. Transdifferentiation of pancreatic cells to hepatocytes can be induced by conversion of a pancreatic exocrine cell line, AR42J-B13, to hepatocytes by treatment with the synthetic glucocorticoid dexamethasone and oncostatin M, a member of the interleukin-6 family [36]. Dexamethasone can also induce pancreas-to-liver conversion in embryonic mouse tissue [36] and adult rat pancreatic cells [37]. While studying the expression profiles of liver-enriched transcription factors in transdifferentiating AR42J-B13 cells, the transcription factor C/EBPβ (CCAAT-enhancer binding protein) was found to be induced in transdifferentiated hepatocytes. Strikingly, transfection of C/EBPβ in B13 cells induced the hepatic phenotype, whereas LIP (liver inhibitory protein), the dominant-negative form of C/EBPβ, inhibited transdifferentiation [36]. The results suggest members of the C/EBP family of transcription factors are candidates for distinguishing liver and pancreas during development [38]. The exact role of C/EBPs in liver development is yet to be clarified. The ability to produce transdifferentiated hepatocytes offers an alternative method to study liver function in long-term cultures, as these show significant functional similarities with differentiated hepatocytes [39, 40].

Transdifferentation in the liver: hepatocyte to bile duct

Liver damage and chronic liver disease can lead to an overgrowth of intrahepatic biliary tissue associated with fibrosis and inflammation [41]. The emerging duct tissue might originate from extensive proliferation of pre-existing bile ducts, liver-specific stem cells or, as shown recently, by transdifferentiation of hepatocytes. Nishikawa et al. reported that cultured rat hepatocytes can convert to CK19-positive intra-

hepatic bile duct cells in presence of insulin and EGF (Fig. 3) [42]. During this transition, ligands (jagged) and target genes (hairy and enhancer of split, Hes) of the notch/delta pathway were upregulated, as seen during normal bile duct development [43]. The hepatobiliary conversion was inhibited by dexamethasone and tyrosine phosphorylation inhibitors. The latter act by EGF signalling downstream of targets MEK1 and PI3. Nishikawa and colleagues suggested that hepatobiliary conversion is an example of direct transdifferentation, as hepatoblast markers (notch ligand 'delta-like') were not expressed throughout, suggesting the absence of transitional cell states. It is unlikely that contaminating oval cells account for the emerging biliary cells, as the same results were obtained in hepatocyte cultures from c-kit (a receptor for stem cell factor, SCF) mutant rats in which the number of oval cells is reduced [44]. Oval cells are progenitor cells assumed to develop from hepatic stem cells and have the potential to differentiate into hepatocytes and bile duct cells.

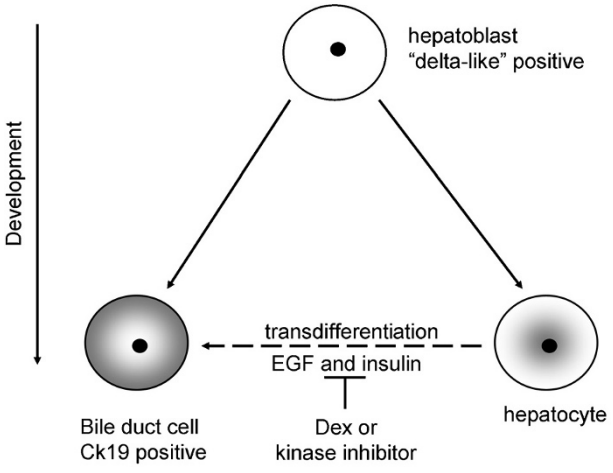

Figure 3. Development and transdifferentiation of hepatocytes to intrahepatic bile duct cells. Hepatocytes convert to cytokeratin 19-positive bile duct cells in the presence of insulin and epidermal growth factor (EGF). Transdifferentiation depends on EGF because inhibition of mitogen-activated protein kinase kinase 1, and phosphatidylinositol 3-kinase prevents the conversion.

Cdx2 – a mediator of gut development and Barrett's metaplasia

In Barrett's oesophagus, squamous epithelium at the lower end of the oesophagus is replaced by epithelium resembling gastric or intestinal mucosa [6]. This metaplasia is proposed to be triggered by reflux of acid and bile from the stomach and duodenum into the oesophagus. The development of Barrett's metaplasia predisposes to oesophageal adenocarcinoma [7]. Although the molecular basis of Barrett's is unclear, recent experiments have suggested a role of the caudal type homeobox transcription factor (Cdx2). Cdx2 is normally expressed in the posterior gut endoderm during development and is a key regulator of intestinal differentiation. *Cdx2* knockout in mice is lethal [45]. Heterozygous *Cdx2* mice are viable, but develop intestinal lesions of esophageal- and forestomach-like keratinizing stratified squamous epithelium due to haploinsufficiency of Cdx2 [46]. This suggests Cdx2 may direct endodermal differentiation towards the caudal phenotype. Interestingly, Cdx2 is upregulated in Barrett's and may therefore be an important factor in the conversion to an intestinal cell type [47]. Indeed, ectopic gastric expression of Cdx2 under the control of a H^+/K^+ ATPase β-subunit promoter brings about intestinal metaplasia [48]. As this proton pump is active after birth, metaplasic cells are likely to have arisen by true transdifferentation from differentiated epithelium rather than from undifferentiated cells that express Cdx2. Using the same transgenic model, Mutoh et al. also showed that long-term intestinal metaplasia induced invasive gastric carcinoma [49]. Experimental modelling of Barrett's metaplasia by overexpression of effector candidate genes like *Cdx2* may elucidate the molecular pathway and enable the discovery of inhibitors for therapeutic purposes. We have recently established two systems to culture embryonic mouse oesophageal tissue that will facilitate drug discovery [50, 51]. Moreover, Fitzgerald and colleagues have suggested the intriguing possibility that bile acids may be acting via induction of retinoic acid, a well-known differentiating agent [52]. Addition of exogenous retinoic acid in turn induces differentiation towards a columnar phenotype [52]. The authors also propose that the source of the cells generating the columnar epithelial phenotype is from mesenchymal cells present in the oesophageal stroma, but this may not constitute an example of transdifferentiation.

Squamous metaplasia in the uterine epithelium

Diethylstilbestrol (DES) is a non-steroidal synthetic oestrogen which was originally administered to pregnant women to prevent miscarriage, but was banned due to association with vaginal adenocarcinoma in their female offspring [53]. Female embryos *in utero* exposed to DES may develop vaginal clear cell adenocarcinoma in later life and commonly display structural malformation of the uterus and cervix and cytological abnormalities, including growth of benign glands in the vagina (vaginal adenosis) [54]. Additionally, squamous metaplasia is observed in the uterus and cervix, which is thought to predispose to

neoplasia [55]. DES has a high binding affinity to the oestrogen receptor α (ERα) and can effectively trigger the oestrogen signalling pathway. Perinatal exposure to DES (in the mouse during the first 5 days after birth) can presumably disturb the expression of transcription factors crucial for the correct proliferation and cytodifferentiation of the female reproductive tract [53]. Recently, Huang and co-workers suggested a molecular basis for uterine squamous metaplasia [56]. Neonatal mice treated with DES showed decreased proliferation of the uterine luminal epithelium and abnormal differentiation indicated by the presence of stratified epithelial markers (small proline-rich proteins, Sprr2a and Sprr2f) and an epidermal keratin (K2.16) which are not expressed in the normal uterine epithelium. Conversion to this unusual cell type occurs due to DES-mediated down-regulation of the homeobox transcription factor Msx2, which is critical for normal uterine cytodifferentiation. Ultimately, it remains to be clarified whether the molecular basis of DES-induced human pathology is similar to the proposed murine model.

Conclusions

The cellular and molecular basis of metaplasia and transdifferentiation is now quite well understood in some cases and may lead to the development of selective therapies to block malignant tissue cell conversions as well as provide cellular therapies. Transdifferentiated cells can be used in the treatment of degenerative disorders as an alternative to differentiated cells induced from embryonic and adult stem cells. As it is the principal molecular components of normal tissue development that mediate metaplasic cell-type conversions, studying metaplasia is an alternative to the direct analysis of embryonic development.

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1 Slack, J. M. and Tosh, D. (2001) Transdifferentiation and metaplasia – switching cell types. Curr. Opin. Genet. Dev. 11, 581–586.
2 Tosh, D. and Slack, J. M. (2002) How cells change their phenotype. Nat. Rev. Mol. Cell Biol. 3, 187–194.
3 Eguchi, G. and Kodama, R. (1993) Transdifferentiation. Curr. Opin. Cell Biol. 5, 1023–1028.
4 Tsonis, P. A. and Del Rio-Tsonis, K. (2004) Lens and retina regeneration: transdifferentiation, stem cells and clinical applications. Exp. Eye Res. 78, 161–172.
5 Beresford, W. A. (1990) Direct transdifferentiation: can cells change their phenotype without dividing? Cell Differ. Dev. 29, 81–93.
6 Sharma, P., McQuaid, K., Dent, J., Fennerty, M. B., Sampliner, R., Spechler, S., Cameron, A., Corley, D., Falk, G., Goldblum, J. et al. (2004) A critical review of the diagnosis and management of Barrett's esophagus: the AGA Chicago Workshop. Gastroenterology 127, 310–330.
7 Haggitt, R. C., Tryzelaar, J., Ellis, F. H. and Colcher, H. (1978) Adenocarcinoma complicating columnar epithelium-lined (Barrett's) esophagus. Am. J. Clin. Pathol. 70, 1–5.
8 Slack, J. M. (1995) Developmental biology of the pancreas. Development 121, 1569–1580.
9 Hezel, A. F., Kimmelman, A. C., Stanger, B. Z., Bardeesy, N. and DePinho, R. A. (2006) Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev. 20, 1218–1249.
10 Lardon, J. and Bouwens, L. (2005) Metaplasia in the pancreas. Differentiation 73, 278–286.
11 Wagner, M., Luhrs, H., Kloppel, G., Adler, G. and Schmid, R. M. (1998) Malignant transformation of duct-like cells originating from acini in transforming growth factor transgenic mice. Gastroenterology 115, 1254–1262.
12 Gu, D. and Sarvetnick, N. (1993) Epithelial cell proliferation and islet neogenesis in IFN-g transgenic mice. Development 118, 33–46.
13 Miyatsuka, T., Kaneto, H., Shiraiwa, T., Matsuoka, T. A., Yamamoto, K., Kato, K., Nakamura, Y., Akira, S., Takeda, K., Kajimoto, Y. et al. (2006) Persistent expression of PDX-1 in the pancreas causes acinar-to-ductal metaplasia through Stat3 activation. Genes Dev. 20, 1435–1440.
14 Means, A. L., Meszoely, I. M., Suzuki, K., Miyamoto, Y., Rustgi, A. K., Coffey, R. J., Jr., Wright, C. V., Stoffers, D. A. and Leach, S. D. (2005) Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. Development 132, 3767–3776.
15 Rooman, I., Heremans, Y., Heimberg, H. and Bouwens, L. (2000) Modulation of rat pancreatic acinoductal transdifferentiation and expression of PDX-1 in vitro. Diabetologia 43, 907–914.
16 Jonsson, J., Carlsson, L., Edlund, T. and Edlund, H. (1994) Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 371, 606–609.
17 Kawaguchi, Y., Cooper, B., Gannon, M., Ray, M., MacDonald, R. J. and Wright, C. V. (2002) The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. Nat. Genet. 32, 128–134.
18 Delacour, A., Nepote, V., Trumpp, A. and Herrera, P. L. (2004) Nestin expression in pancreatic exocrine cell lineages. Mech. Dev. 121, 3–14.
19 Selander, L. and Edlund, H. (2002) Nestin is expressed in mesenchymal and not epithelial cells of the developing mouse pancreas. Mech. Dev. 113, 189–192.
20 Bouwens, L. (1998) Cytokeratins and cell differentiation in the pancreas. J. Pathol. 184, 234–239.
20a World Health Organization (2007) Diabetes, Fact sheet no. 312. http://www.who.int/mediacentre/factsheets/312/en/ [accessed 28 October 2007].
21 Samson, S. L. and Chan, L. (2006) Gene therapy for diabetes: reinventing the islet. Trends Endocrinol. Metab. 17, 92–100.
22 Horb, M. E., Shen, C. N., Tosh, D. and Slack, J. M. (2003) Experimental conversion of liver to pancreas. Curr. Biol. 13, 105–115.
23 Ber, I., Shternhall, K., Perl, S., Ohanuna, Z., Goldberg, I., Barshack, I., Benvenisti-Zarum, L., Meivar-Levy, I. and Ferber, S. (2003) Functional, persistent, and extended liver to pancreas transdifferentiation. J. Biol. Chem. 278, 31950–31957.
24 Ferber, S., Halkin, A., Cohen, H., Ber, I., Einav, Y., Goldberg, I., Barshack, I., Seijffers, R., Kopolovic, J., Kaiser, N. et al. (2000) Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat Med 6, 568–572.
25 Kaneto, H., Nakatani, Y., Miyatsuka, T., Matsuoka, T. A., Matsuhisa, M., Hori, M. and Yamasaki, Y. (2005) PDX-1/VP16 fusion protein, together with NeuroD or Ngn3, markedly induces insulin gene transcription and ameliorates glucose tolerance. Diabetes 54, 1009–1022.

26 Rajagopal, J., Anderson, W. J., Kume, S., Martinez, O. I. and Melton, D. A. (2003) Insulin staining of ES cell progeny from insulin uptake. Science 299, 363.
27 Deutsch, G., Jung, J., Zheng, M., Lora, J. and Zaret, K. S. (2001) A bipotential precursor population for pancreas and liver within the embryonic endoderm. Development 128, 871–881.
28 Barbosa, J., Dockerty, M. and Waugh, J. (1946) Pancreatic heterotopia: review of the literature and report of 41 authenticated surgical cases, of which 25 were clinically relevant. Surg. Gynecol. Obstet. 82, 527–542.
29 Dutton, J. R., Chillingworth, N. L., Eberhard, D., Brannon, C. R., Hornsey, M. A., Tosh, D. and Slack, J. M. (2006) {beta} cells occur naturally in extrahepatic bile ducts of mice. J. Cell Sci. 120 (Pt 2), 239–245.
30 Sumazaki, R., Shiojiri, N., Isoyama, S., Masu, M., Keino-Masu, K., Osawa, M., Nakauchi, H., Kageyama, R. and Matsui, A. (2004) Conversion of biliary system to pancreatic tissue in Hes1-deficient mice. Nat. Genet. 36, 83–87.
31 Burke, Z. D., Shen, C. N. and Tosh, D. (2004) Bile ducts as a source of pancreatic beta cells. Bioessays 26, 932–937.
32 Rao, M. S., Dwivedi, R. S., Subbarao, V., Usman, M. I., Scarpelli, D. G., Nemali, M. R., Yeldandi, A., Thangada, S., Kumar, S. and Reddy, J. K. (1988) Almost total conversion of pancreas to liver in the adult rat: a reliable model to study transdifferentiation. Biochem. Biophys. Res. Commun. 156, 131–136.
33 Tosh, D., Shen, C. N., Alison, M. R., Sarraf, C. E. and Slack, J. M. (2007) Copper deprivation in rats induces islet hyperplasia and hepatic metaplasia in the pancreas. Biol. Cell 99, 37–44.
34 Krakowski, M. L., Kritzik, M. R., Jones, E. M., Krahl, T., Lee, J., Arnush, M., Gu, D. and Sarvetnick, N. (1999) Pancreatic expression of keratinocyte growth factor leads to differentiation of islet hepatocytes and proliferation of duct cells. Am. J. Pathol. 154, 683–691.
35 Paner, G. P., Thompson, K. S. and Reyes, C. V. (2000) Hepatoid carcinoma of the pancreas. Cancer 88, 1582–1589.
36 Shen, C. N., Slack, J. M. and Tosh, D. (2000) Molecular basis of transdifferentiation of pancreas to liver. Nat. Cell Biol. 2, 879–887.
37 Lardon, J., De Breuck, S., Rooman, I., Van Lommel, L., Kruhoffer, M., Orntoft, T., Schuit, F. and Bouwens, L. (2004) Plasticity in the adult rat pancreas: transdifferentiation of exocrine to hepatocyte-like cells in primary culture. Hepatology 39, 1499–1507.
38 Westmacott, A., Burke, Z. D., Oliver, G., Slack, J. M. and Tosh, D. (2006) C/EBPalpha and C/EBPbeta are markers of early liver development. Int J Dev Biol 50, 653–657.
39 Burke, Z. D., Shen, C. N., Ralphs, K. L. and Tosh, D. (2006) Characterization of liver function in transdifferentiated hepatocytes. J. Cell Physiol. 206, 147–159.
40 Kurash, J. K., Shen, C. N. and Tosh, D. (2004) Induction and regulation of acute phase proteins in transdifferentiated hepatocytes. Exp. Cell Res. 292, 342–358.
41 Sirica, A. E. (1995) Ductular hepatocytes. Histol. Histopathol. 10, 433–456.
42 Nishikawa, Y., Doi, Y., Watanabe, H., Tokairin, T., Omori, Y., Su, M., Yoshioka, T. and Enomoto, K. (2005) Transdifferentiation of mature rat hepatocytes into bile duct-like cells in vitro. Am. J. Pathol. 166, 1077–1088.
43 Lemaigre, F. and Zaret, K. S. (2004) Liver development update: new embryo models, cell lineage control, and morphogenesis. Curr. Opin. Genet. Dev. 14, 582–590.
44 Matsusaka, S., Tsujimura, T., Toyosaka, A., Nakasho, K., Sugihara, A., Okamoto, E., Uematsu, K. and Terada, N. (1999) Role of c-kit receptor tyrosine kinase in development of oval cells in the rat 2-acetylaminofluorene/partial hepatectomy model. Hepatology 29, 670–676.
45 Chawengsaksophak, K., James, R., Hammond, V. E., Kontgen, F. and Beck, F. (1997) Homeosis and intestinal tumours in Cdx2 mutant mice. Nature 386, 84–87.
46 Beck, F., Chawengsaksophak, K., Waring, P., Playford, R. J. and Furness, J. B. (1999) Reprogramming of intestinal differentiation and intercalary regeneration in Cdx2 mutant mice. Proc. Natl. Acad. Sci. USA 96, 7318–7323.
47 Eda, A., Osawa, H., Satoh, K., Yanaka, I., Kihira, K., Ishino, Y., Mutoh, H. and Sugano, K. (2003) Aberrant expression of CDX2 in Barrett's epithelium and inflammatory esophageal mucosa. J. Gastroenterol. 38, 14–22.
48 Mutoh, H., Hakamata, Y., Sato, K., Eda, A., Yanaka, I., Honda, S., Osawa, H., Kaneko, Y. and Sugano, K. (2002) Conversion of gastric mucosa to intestinal metaplasia in Cdx2-expressing transgenic mice. Biochem. Biophys. Res. Commun. 294, 470–479.
49 Mutoh, H., Sakurai, S., Satoh, K., Tamada, K., Kita, H., Osawa, H., Tomiyama, T., Sato, Y., Yamamoto, H., Isoda, N. et al. (2004) Development of gastric carcinoma from intestinal metaplasia in Cdx2-transgenic mice. Cancer Res. 64, 7740–7747.
50 Quinlan, J. M., Yu, W. Y., Hornsey, M. A., Tosh, D. and Slack, J. M. (2006) In vitro culture of embryonic mouse intestinal epithelium: cell differentiation and introduction of reporter genes. BMC Dev. Biol. 6, 24.
51 Yu, W. Y., Slack, J. M. and Tosh, D. (2005) Conversion of columnar to stratified squamous epithelium in the developing mouse oesophagus. Dev. Biol. 284, 157–170.
52 Fitzgerald, R. C. (2006) Molecular basis of Barrett's oesophagus and oesophageal adenocarcinoma. Gut 55, 1810–1820.
53 Miller, K. P., Borgeest, C., Greenfeld, C., Tomic, D. and Flaws, J. A. (2004) In utero effects of chemicals on reproductive tissues in females. Toxicol. Appl. Pharmacol. 198, 111–131.
54 Herbst, A. L. (1981) Clear cell adenocarcinoma and the current status of DES-exposed females. Cancer 48, 484–488.
55 Hatch, E. E., Herbst, A. L., Hoover, R. N., Noller, K. L., Adam, E., Kaufman, R. H., Palmer, J. R., Titus-Ernstoff, L., Hyer, M., Hartge, P. et al. (2001) Incidence of squamous neoplasia of the cervix and vagina in women exposed prenatally to diethylstilbestrol (United States). Cancer Causes Control 12, 837–845.
56 Huang, W. W., Yin, Y., Bi, Q., Chiang, T. C., Garner, N., Vuoristo, J., McLachlan, J. A. and Ma, L. (2005) Developmental diethylstilbestrol exposure alters genetic pathways of uterine cytodifferentiation. Mol. Endocrinol. 19, 669–682.