The role of Cdx2 in Barrett's metaplasia

Benjamin J. Colleypriest*[1], J. Mark Farrant†, Jonathan M.W. Slack*[2] and David Tosh*

*Centre for Regenerative Medicine, Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, U.K. and †Department of Gastroenterology, Royal United Hospital, Combe Park, Bath BA13NG, U.K.

Abstract

Metaplasia (or transdifferentiation) is defined as the transformation of one tissue type to another. Clues to the molecular mechanisms that control the development of metaplasia are implied from knowledge of the transcription factors that specify tissue identity during normal embryonic development. Barrett's metaplasia describes the development of a columnar/intestinal phenotype in the squamous oesophageal epithelium and is the major risk factor for oesophageal adenocarcinoma. This particular type of cancer has a rapidly rising incidence and a dismal prognosis. The homoeotic transcription factor Cdx2 (*Caudal*-type homeobox 2) has been implicated as a master switch gene for intestine and therefore for Barrett's metaplasia. Normally, *Cdx2* expression is restricted to the epithelium of the small and large intestine. Loss of Cdx2 function, or conditional deletion in the intestine, results in replacement of intestinal cells with a stratified squamous phenotype. In addition, Cdx2 is sufficient to provoke intestinal metaplasia in the stomach. In the present paper, we review the evidence for the role of Cdx2 in the development of Barrett's metaplasia.

Metaplasia

Metaplasia is a pathological term which describes the phenomenon of a cell or tissue acquiring a different phenotype during postnatal life [1]. It is not uncommon and can result in the acquisition of a focus of ectopic tissue. Metaplasia can arise because of a change in the phenotype of a tissue-specific stem cell (i.e. the conversion of one tissue-specific stem cell into another), a differentiated cell or any of the intermediate phenotypes. Stem cells are defined as undifferentiated cells that can both self-renew and generate specialized (functional) cell types [2]. Adult stem cells generally populate one tissue type, e.g. an intestinal stem is restricted to progeny that can form the four intestinal cell types. There are numerous examples of metaplasia occurring in most tissues of the body and it is especially common in epithelia, often arising in the context of regeneration. Metaplasia generally occurs between cell types that arise from neighbouring regions of the developing embryo. This suggests that neighbouring tissues may differ in the expression of one or a few transcription factors (so-called master switch genes). Although metaplasia may arise in tissues that are adjacent in the embryo, for example gastric metaplasia developing in the duodenum [3], the developmental association is not always clear from the adult anatomy. For example, cystitis glandularis is a pathological term for patches of colonic mucosa that are acquired in the bladder [4]. These regions represent distinct organs in the adult, but in the embryo, the bladder develops from a neighbouring region of hindgut and may explain the propensity for these tissues to interconvert.

Key words: Barrett's metaplasia, *Cdx2*, oesophageal adenocarcinoma, pathogen-associated molecular pattern (PAMP), proton pump inhibitor (PPI).

Abbreviations used: E, embryonic day; LPS, lipopolysaccharide; OAC, oesophageal adenocarcinoma; PAMP, pathogen-associated molecular pattern; PPI, proton pump inhibitor.

[1]**To whom correspondence should be addressed (email Bcolleypriest@doctors.net.uk)**.

[2]**Present address:** Stem Cell Institute, University of Minnesota, MTRF, 2001 6th Street SE, Minneapolis, MN 55455, U.S.A.

Barrett's metaplasia and OAC (oesophageal adenocarcinoma)

Barrett's metaplasia describes the pathological condition characterized by a phenotypic switch in the epithelial cells of the distal oesophagus from the normal stratified squamous mucosa to an intestinal columnar type [5]. Barrett's metaplasia is synonymous with Barrett's oesophagus and is considered to be an example of intestinal metaplasia. All four intestinal cell types (enterocytes, goblet cells, Paneth cells and enteroendocrine cells) can be represented in Barrett's metaplasia, but Paneth cells are more rarely found and the condition may also be described as incomplete intestinal metaplasia. Barrett's metaplasia may arise in the context of chronic inflammation and can be provoked in the oesophagus of the dog and rat with surgical procedures that induce reflux [6,7]. Although Barrett's metaplasia develops in the context of chronic reflux, it is an inherently asymptomatic condition that is often detected incidentally on endoscopy [8].

For the diagnosis of Barrett's metaplasia, the majority of countries, but not the U.K. (which follows guidelines of the British Society of Gastroenterology [9]) require specialized intestinal cells in the oesophagus. For the purposes of the present review, Barrett's metaplasia will be considered as an intestinal metaplasia. Barrett's metaplasia is an important pathology because it is the major risk factor for, and only known precursor to, OAC. The risk of OAC is increased between 30–125 times with the development of Barrett's metaplasia [10], which equates to a 1 in 20 lifetime risk [11].

The prevalence of Barrett's metaplasia in the population is unknown, but studies suggest that it is increasing [12]. In a Scottish study, the prevalence rose drastically from 1

in 1000 endoscopies in the 1980s to 60 in 1000 in the late 1990s [13]. An increase in incidence from 14.3 to 23.1 cases per 100000 people between 1997 and 2002 was noted in a Dutch study. Given that during the study period the absolute number of endoscopies decreased, the number of cases of Barrett's metaplasia per 1000 endoscopies rose from 19 to 40 [12].

Management of Barrett's metaplasia

The risk of progression to OAC from Barrett's metaplasia varies in the literature, and smaller studies report higher risk [10]. The majority of recent studies suggest an annual risk of progression of approx. 0.5–1 % [14–16] and U.K. values concur, with an incidence of 0.9 % [16]. OAC represents a major problem in the developing world with an incidence that has increased 500 % over the last 25 years and is highest in the U.K. [17,18]. Despite the increase in diagnosis, the prognosis remains poor.

At present, there is no long-term curative treatment for Barrett's metaplasia, and surveillance programmes are advised to detect histological evidence of progression towards cancer. The rationale for surveying this high-risk population relates to the survival statistics for oesophageal cancer, which closely follows staging at diagnosis. Pharmacological acid suppression, usually with PPIs (proton pump inhibitors), currently provides the mainstay of medical treatment for Barrett's metaplasia. PPIs provide an effective treatment for reflux symptoms, oesophagitis and stricture prevention; however, a complete reversal of Barrett's metaplasia is not seen, and the evidence for preventing the progression to OAC is conflicting [19–21]. There is a theoretical risk of cancer with long-term PPI use related to increased serum gastrin levels that develop in a proportion of patients. Addition of gastrin increases proliferation of an OAC cell line (OE33) [22]. The use of ablative techniques to treat Barrett's metaplasia, such as photodynamic therapy and radiofrequency ablation, are becoming more widespread, but are confined to the treatment of dysplastic Barrett's metaplasia. Radiofrequency ablation demonstrates the best success rates for treating dysplasia and does provoke the regeneration of normal squamous mucosa [23]. In a recent study, complete eradiation of high-grade dysplasia was evident in 81 % patients and of intestinal metaplasia in 77.4 % of the patients treated [23]. Radiofrequency ablation may be appropriate for the long-term eradication of non-dysplastic Barrett's metaplasia, but such studies are required.

OAC arises in metaplastic columnar epithelium in the distal oesophagus, which can be of three types: gastric–fundal, cardiac or intestinal [24,25]. The majority of cases of OAC have been considered to arise within intestinal metaplasia [26]. However, recent publications have questioned this by demonstrating OAC in metaplastic epithelium without specialized cells and DNA abnormalities, such as aneuploidy, in gastric cardia mucosa [27,28]. The pathogenesis of Barrett's metaplasia revolves around inflammation arising from the reflux of acid and bile provoking the oesophageal squamous cells to be replaced with columnar epithelium [29], although the mechanism responsible for this is not fully understood.

Cancer of the oesophagus often remains asymptomatic until late in the disease and consequently carries a poor prognosis, with a 5-year survival rate of between 5 and 15 % [18,30–32]. An understanding of the molecular steps that result in Barrett's metaplasia may offer the potential for targeted interventions at a metaplastic or even pre-metaplastic stage. If Barrett's metaplasia can be reverted to normal squamous epithelium with targeted molecular therapy, possibly in combination with ablative techniques, then the increased risk of OAC may be negated.

Tissue type specification during normal development

In order to determine the molecular mechanisms that are responsible for metaplasia, it is important to understand how tissue types are specified during normal development. Embryogenesis involves the tightly regulated temporal and spatial hierarchical expression of genes that determine specialized cell types. A specific set of transcription factors and signalling molecules are important regulators of tissue type during embryogenesis of the gastrointestinal tract.

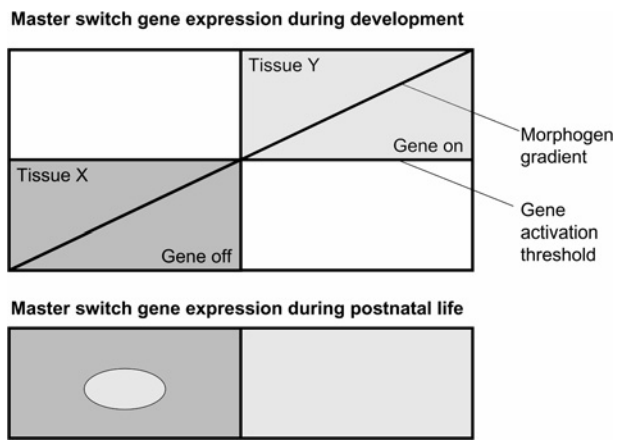
Metaplastic tissue may arise because of an altered pattern of expression of genes that determine tissue types during development [2,33]. Key tissue-type-regulatory genes that are misexpressed in metaplasia are referred to as homoeotic or master switch genes [33]. Development of the various organs of the gut during embryogenesis results from a common sheet of cells that differentiate in response to soluble factors or morphogens such as FGFs (fibroblast growth factors), BMP (bone morphogenetic protein), SHH (Sonic Hedgehog) or RA (retinoic acid). Transcription factors, encoded by master switch genes, are activated or repressed accordingly as different tissues are specified. Master switch genes can dictate antero–posterior body patterning, as in the case of Hox genes, or specific tissue types, for example *Cdx2* (*Caudal*-type homeobox 2) specifying intestine [34]. It is hypothesized that metaplastic tissue is distinguished from the surrounding tissue by the state of just one or a few transcription factors because it commonly represents a transition between embryologically related (neighbouring) tissues. If this is true, then ectopic expression (or repression) of a master switch gene in postnatal life could be responsible for the development of metaplasia (Figure 1). Some of the transcription factors that are important in the development of oesophageal, stomach and intestine are shown in Figure 2.

Hox and ParaHox genes

The Homeobox gene family is a highly conserved set of genes containing a helix–turn–helix DNA-binding motif and convey homoeotic functions. A subgroup of Homeobox genes, the Hox genes, control antero–posterior patterning of the developing embryo. Hox genes encode transcription factors that regulate genes important for body patterning and cell fate determination. The 39 human Hox genes

Figure 1 | A model for the development of metaplasia based on transcription factor misexpression

During development tissue X (dark grey) becomes tissue Y (light grey) in response to activation of a transcription factor dependent on morphogen concentration. If this transcription factor is ectopically expressed in postnatal life within tissue X, a focus of metaplastic tissue Y develops.

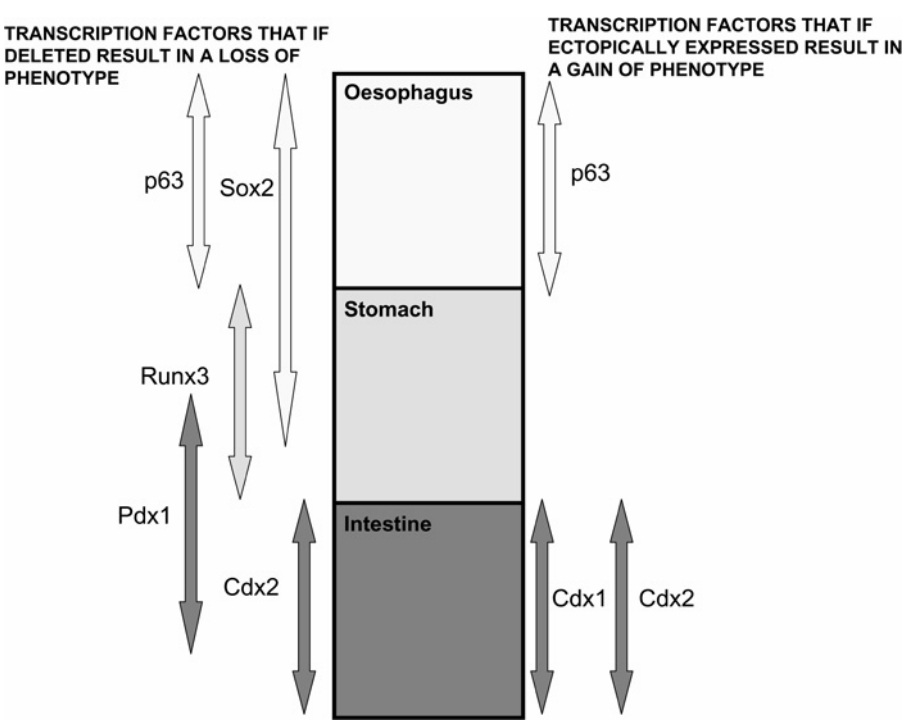


are organized into 13 paralogous groups on four separate chromosomes [35]: *HOXA*, *HOXB*, *HOXC* and *HOXD* on chromosomes 7, 17, 12 and 2 respectively. These genes are expressed in a co-linear pattern along the length of the developing embryo and impart a Hox code to each region [36,37]. Genes at one end of the Hox cluster are expressed and pattern the anterior region, whereas genes at the other end pattern the posterior embryo. The Hox gene cluster exhibits a spatial co-linearity, such that genes are activated in sequence from one end to the other. Consequently, the organization of the Hox gene cluster tightly regulates the anterior–posterior axis of animals.

A paralogous evolutionary sister of the Hox genes, the ParaHox cluster, consists of the *CDX2*, *PDX1* (pancreatic and duodenal homeobox 1) and *GSH* (genomic screened homeobox) genes [38]. The *CDX2* gene, located on chromosome 13 in humans and chromosome 5 in the mouse, is a member of this ParaHox cluster. *CDX2* belongs to a class of genes encoding transcription factors homologous with the *Drosophila* gene *Caudal* [39]. *Caudal* is a homeobox gene with roles in early patterning of the posterior segment and later development of the hindgut [40]. In mice and

Figure 2 | Expression of transcription factors integral to tissue specification during development of the oesophagus, stomach and intestine

Transcription factors may be important in loss-of-phenotype or gain-of-phenotype (or both) scenarios when misexpressed. *In vivo*, loss of expression of *p63*, *Sox2* [SRY (sex-determining region Y) box 2], *Runx3* (runt-related transcription factor 3), *Pdx1* or *Cdx2* in its native tissue results in a loss of the normal phenotype within that epithelium. For example, loss of either p63 or Sox2 results in replacement of the squamous oesophagus with columnar tissue. Ectopic expression of p63, Cdx1 or Cdx2 in neighbouring tissues during development results in the replacement of this tissue with the type normally specified by the transcription factor. For example, ectopic *Cdx1* or *Cdx2* expression in the stomach results in a gastric intestinal metaplasia.



humans, there are three *Caudal* homologues, *CDX1*, *CDX2* and *CDX4*, of which only *CDX1* and *CDX2* have roles in development of the gastrointestinal tract. In the mouse embryo, expression of *Cdx2* is first evident at E (embryonic day) 3.5 and is confined to the trophectoderm, and persists in the extra-embryonic ectoderm. From E8.5, *Cdx2* is expressed in the posterior gut endoderm, neural tube and tail bud [41]. By E12.5, *Cdx2* is restricted to the endoderm of the gut [42] and its expression increases significantly during the transformation of endoderm into a columnar epithelium (E14–E17) [43]. Two separate transgenic models demonstrate the necessity of *Cdx2* for normal intestinal development, suggesting a role as the master switch gene in this tissue. First, mice with only one wild-type allele for *Cdx2* develop squamous metaplasia within the intestine and colon [44]. These areas do not express Cdx2 protein and suggest that the loss of expression provokes the intestinal tissue to develop into oesophageal-like tissue. Secondly, this essential role in intestine specification is confirmed by conditional deletion of *Cdx2* in the developing endoderm. Intestinal epithelium is replaced by squamous mucosa expressing oesophageal-specific genes in mice when *Cdx2* is deleted under the control of the *FoxA3* (forkhead box A3) promoter [34]. Furthermore, *Cdx2* has been shown to regulate the expression of many intestinal, e.g. *ALPI* (alkaline phosphatase), *TFF3* (trefoil factor 3), *SI* (sucrase-isomaltase), *MUC2* (mucin 2), *VIL1* (villin 1) and *LCH* (lactase–phlorizin hydrolase) [45–50]. The critical role of *Cdx2* in intestine specification during development raises the possibility that it may be involved in the development of intestinal metaplasia in non-intestinal tissues.

Cdx2 and intestinal metaplasia

CDX2 is circumstantially linked to intestinal metaplasia because it is expressed in a multitude of tissues that display intestinal metaplasia, such as oesophagus [51], stomach [52], gall bladder [53], biliary tree [54], urinary tract [55], bladder [56], liver [57] and pancreas [58]. The finding of *CDX2* in normal squamous epithelium proximal to the Barrett's metaplasia segment in one-third of patients [59] suggests that its expression precedes the switch in phenotype and may be sufficient to provoke intestinal metaplasia.

Recent publications provide evidence for a link between the innate immune system and *Cdx2* regulation (reviewed in [60]). In cholangiocytes, PAMPs (pathogen-associated molecular patterns), such as LPS (lipopolysaccharide), induce *Cdx2* and *Muc2* via an NF-κB (nuclear factor κB)-dependent mechanism [61–63]. PAMPs are recognized by PRRs (pattern-recognition receptors) such as TLRs (Toll-like receptors) and activate the innate immune system. Murine BECs (biliary epithelial cells) up-regulate the intestinal and gastric mucins *Muc2* and *Muc5ac* expression in response to the Gram-negative bacterial component LPS [63]. These observations provide a potential mechanism to link the main protagonist of Barrett's metaplasia, inflammation and the induction of *Cdx2*.

Figure 3 | The effect of ectopic *Cdx2* expression in different models of gastrointestinal epithelium

The effect of ectopic *Cdx2* expression within cell lines, primary cells and transgenic animal. The anatomical region represented by each model has been highlighted. Intestinal genes that have been provoked by ectopic *Cdx2* expression have been recorded. No intestinal genes were induced in the two models marked with an asterisk (*).

Cell lines		Primary cells	Transgenic
HET-1A (oesophageal immortalised) Muc2, villin, sucrase isomaltase and K20	Oesophagus	Rat keratinocytes Muc2	**K14 promoter *** No intestinal genes
AGS cell line (Gastric adenocarcinoma) Trefoil factor 3	Stomach		**FoxA3 promoter** Goblet, enteroendocrine and enterocytes **H⁺ pump promoter** Goblet, enteroendocrine and enterocytes
Rat embryonic intestine Sucrase isomaltase **Human embryonic intestine *** No intestinal genes	Intestine		

Cdx2 is sufficient to provoke functional intestinal development in the stomach of transgenic mice. Silberg et al. [64] colleagues directed *Cdx2* expression to the stomach using *cis*-regulatory elements of the *FoxA3* promoter [64], resulting in gastric intestinal metaplasia. Mutoh et al. [65] achieved similar results using the H^+/K^+ ATPase promoter to drive gastric *Cdx2* expression. Interestingly, the neo-intestinal mucosa is functional and rescues these transgenic mice from extensive intestinal resection [66].

This indicates that *Cdx2* overexpression can drive intestine formation in the stomach of the fetus, although a recent transgenic mouse in which oesophageal *Cdx2* is driven from the K14 squamous epithelial specific promoter lacked any expression of intestinal genes [67]. There is also conflicting evidence to address whether Cdx2 is sufficient to provoke an intestinal metaplasia in postnatal oesophageal cells (Figure 3). Overexpression of the cell-cycle regulator, cyclin D1, and demethylating agents are both required in addition to ectopic *CDX2* expression in order to provoke the expression of intestinal genes in immortalized oesophageal cells [68]. However, *Cdx2* expression in primary rat oesophageal cells does provoke the goblet cell marker *Muc2*, and ectopic *Cdx2* expression in HET-1A cells induces *Muc2*, *vil1*, *SI* and *K20* (cytokeratin 20) [69,70]. Interestingly, the effect of Cdx2 on rat and human embryonic intestinal cells is different. Whereas *Cdx2* provokes *SI* in rat IEC-6 cells, it does not induce intestinal differentiation in HIEC (human intestinal epithelial crypt) cells [71,72]. Although Barrett's metaplasia is an acquired phenomenon, the majority of evidence concerning *Cdx2* overexpression relates to non-physiological models such as cell lines or embryonic tissue. At present, it seems

likely that its expression is necessary for development of Barrett's metaplasia, although it is probably not sufficient on its own to drive the process.

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