

Barrett's Metaplasia

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Modelling Barrett's oesophagus

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Abstract

Barrett's oesophagus is the replacement of normal squamous oesophageal epithelium with an intestinalized columnar epithelium. Although some insight has been gained as to what Barrett's oesophagus is, how this columnar epithelium emerges from within a stratified squamous epithelium remains an unanswered question. We have sought to determine whether oesophageal keratinocytes can be trans-differentiated into Barrett's oesophagus cells. Using an Affymetrix microarray, we found unexpectedly that gene-expression patterns in the Barrett's oesophagus were only slightly more similar to the normal small intestine than they were to the normal oesophagus. Thus gene-expression patterns suggest significant molecular similarities remain between Barrett's oesophagus cells and normal squamous oesophageal epithelium, despite their histological resemblance with intestine. We next determined whether directed expression of intestine-specific transcription factors could induce intestinalization of keratinocytes. Retroviral-mediated Cdx2 (*Caudal*-type homeobox 2) expression in immortalized human oesophageal keratinocytes engineered with human telomerase reverse transcriptase (EPC2-hTERT cells) could be established transiently, but not maintained, and was associated with a reduction in cell proliferation. Co-expression of cyclin D1 rescued proliferation in the Cdx2-expressing cells, but co-expression of dominant-negative p53 did not. Cdx2 expression in the EPC2-hTERT.D1 cells did not induce intestinalization. However, when combined with treatments that induce chromatin remodelling, there was a significant induction of Barrett's oesophagus-associated genes. Studies are ongoing to determine whether other intestinal transcription factors, either alone or in combination, can provoke greater intestinalization of oesophageal keratinocytes. We conclude that, on the basis of gene-expression patterns, Barrett's oesophagus epithelial cells may represent an intermediate between oesophageal keratinocytes and intestinal epithelial cells. Moreover, our findings suggest that it may be possible to induce Barrett's oesophagus epithelial cells from oesophageal keratinocytes by altering the expression of certain critical genes.

Introduction

Barrett's oesophagus occurs at the gastro-oesophageal junction and is the replacement of normal squamous oesophageal epithelium with an intestinalized columnar epithelium. It arises in the setting of chronic acid and bile reflux that results in chronic injury and inflammation in the oesophageal epithelium (Figure 1). Barrett's oesophagus is characterized by the presence of a specialized columnar epithelium with a cellular morphology and gene-expression

pattern typical of intestinal epithelium. Intestinal proteins such as villin, sucrase isomaltase, acidic mucins/MUC2, and the intestine-specific Cdx (*Caudal*-type homeobox) transcription factors Cdx1 and Cdx2, are commonly detected in human Barrett's oesophagus tissues [1]. However, for the pathologist, the key for the diagnosis of Barrett's metaplasia is the presence of intestinal mucin-producing goblet cells [2].

Unless it is associated with symptomatic GORD (gastro-oesophageal reflux disease), Barrett's oesophagus is typically asymptomatic. It is the association with OAC (oesophageal adenocarcinoma) that makes this a clinically significant condition. The progression rate of Barrett's oesophagus to OAC has been estimated to be 0.5–1% per year [3]. Thus only a portion of patients with Barrett's oesophagus will progress

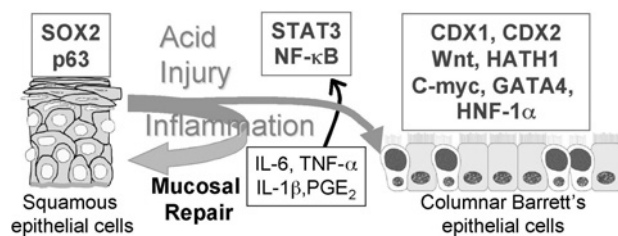
Key words: Barrett's oesophagus, *Caudal*-type homeobox 1 (Cdx1), *Caudal*-type homeobox 2 (Cdx2), c-Myc, EPC2-hTERT cell, microarray.

Abbreviations used: 5-AzaC, 5-aza-2-deoxycytidine; Cdx, *Caudal*-type homeobox; GFP, green fluorescent protein; mATH1, mouse atonal homologue 1; OAC, oesophageal adenocarcinoma; pRb, retinoblastoma protein.

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Figure 1 | Barrett's oesophagus arises in the setting of chronic injury to the oesophagus from gastric and bile acid reflux

Barrett's oesophagus is the replacement of normal squamous oesophageal epithelium with an intestinalized columnar epithelium. HNF-1 α , hepatic nuclear factor 1 α ; IL, interleukin; NF- κ B, nuclear factor κ B; PGE₂, prostaglandin E₂; SOX2, sex-determining region Y box 2; STAT3, signal transducer and activator of transcription 3.



to OAC, complicating the research effort. For this reason, much of the research to date has focused on identifying factors that predict neoplastic progression. Less is known about the molecular mechanisms giving rise to Barrett's oesophagus from normal squamous oesophageal epithelium. One limitation in the study of the genesis and pathobiology of Barrett's oesophagus has been the lack of suitable experimental models. We and others have begun exploring a variety of approaches to model Barrett's oesophagus in the laboratory, and these studies are now yielding important insights into the cell of origin and molecular processes that give rise to Barrett's oesophagus.

Current cell-culture and animal models for Barrett's oesophagus

The primary models used to date have been human Barrett's oesophagus tissue explants and OAC cell lines, with some recent work in primary oesophageal keratinocytes [4–7]. Recently, the identity of several of these common OAC cell lines has been called into question, complicating interpretations of the reported findings [8]. Barrett's oesophagus cell lines have also been developed [9,10]. Although primary Barrett's oesophagus cell lines are useful for studies of the progression from Barrett's oesophagus to OAC, they are less helpful for understanding the genesis of Barrett's oesophagus.

Current animal models for bile and peptic acid reflux disease and Barrett's oesophagus exist in rabbits and rats [11–13]. Two of these models require major animal surgery, with the placement of either an oesophagoduodenostomy or an oesophagojejunostomy in rats [14–16]. The degree of metaplasia that results after surgery is significant, very reminiscent of the human disease. There are several limitations to this approach, however. It is expensive and very operator-dependent (requiring a great deal of small-animal surgical skill), both serving to limit its widespread use. Perhaps most importantly, this approach does not take full advantage of the many transgenic reagents available in mice. For these reasons, it is unlikely the surgical anastomosis model in its present form will be widely utilized.

Gene discovery and Barrett's oesophagus pathogenesis

Transcription factors are likely to be very important contributors to the pathogenesis of Barrett's oesophagus (Figure 1). These proteins bind DNA to regulate gene-expression patterns and can therefore have profound effects on cell differentiation and morphology. In order to identify genes involved in the transdifferentiation process, we performed an Affymetrix microarray analysis on biopsy samples from Barrett's oesophagus without dysplasia and normal oesophagus (from the same patient), as well as small-intestine biopsies from unrelated donors [17]. A Pearson correlation analysis found that the Barrett's oesophagus mRNA expression patterns were more heterogeneous than either of the two normal samples. Moreover, we observed that the Barrett's oesophagus expression profile was only slightly more similar to normal small intestine than it was to normal oesophagus. One interpretation of this finding is that Barrett's oesophagus may be at an intermediate differentiation state between oesophagus and intestine, more so than is suggested by simple histology. Moreover, it suggests, but does not prove, that the oesophageal keratinocyte is the cell of origin for Barrett's oesophagus.

Gene lists were then generated comparing either the Barrett's oesophagus or small intestine with the normal oesophagus profiles. The Barrett's oesophagus samples had only 743 genes that significantly differed from normal oesophagus, whereas small intestine had nearly 3000 genes. However, 583 of the 743 genes were common to both Barrett's oesophagus and the small intestine, implying that this subset of genes is likely to be important for the Barrett's oesophagus cell phenotype. A number of classic Barrett's oesophagus markers including villin, mucin 2, mucin 5AC and defensin α_5 were detected as altered in our gene lists. Importantly, the intestine-specific transcription factor Cdx1 was induced in our Barrett's oesophagus samples, but the homologue Cdx2 and the classic intestinal enzyme and Barrett's oesophagus marker alkaline phosphatase were not. We subsequently demonstrated Cdx2 protein by immunohistochemistry in biopsy samples from the same patients, suggesting that this failure may be due to the insensitivity of the array for this gene product rather than the absence of Cdx2 from the Barrett's oesophagus samples. Finally, a pathway analysis of the genes identified as significantly altered in Barrett's oesophagus identified several genes involved in the c-Myc pathway, including Myc inhibitors and target genes. Together, they suggested that an increase in c-Myc activity may be important for the pathogenesis of Barrett's oesophagus.

Cdx1, Cdx2 and the intestinal cell phenotype

In the intestine, the *Caudal*-related factors Cdx1 and Cdx2 play critical roles in intestinal epithelial development and intestine-specific gene expression [18]. These *Caudal* homologues are very similar structurally and share many overlapping functions. A growing number of genes have been

found to rely upon Cdx1 and Cdx2 for their intestine-specific expression [19–22]. Cdx1 and Cdx2 are also important regulators of intestinal cell to cell adhesion, cell polarity and columnar shape [18,22–26]. Several examples of Cdx1- and Cdx2-mediated columnar morphogenesis have been reported [25,27]. Despite these similarities, only Cdx2 expression is absolutely required for the normal development of the intestinal epithelium; Cdx1 is not [28].

The Cdx1 and Cdx2 expression is normally limited to the small intestine and colon in adults. However, multiple studies confirm ectopic Cdx1 and Cdx2 expression in intestinal metaplasias of the stomach [29–31] and Barrett's oesophagus [30,32,33]. In fact, *CDX2* mRNA expression can be detected in gastric and oesophageal epithelium that is chronically inflamed, preceding the appearance of identifiable intestinal metaplasia [33,34], suggesting that induction of *CDX2* expression may be among the earliest events in the development of intestinal metaplasia. A causal link between Cdx1 and Cdx2 expression and gastric intestinal metaplasia has been established by transgenic expression of Cdx1 or Cdx2 in murine gastric mucosa [35,36]. Gastric intestinal metaplasia was induced by the ectopic expression of these *Caudal* homologues and was marked by the induction of intestinal type goblet cells, and intestinal gene-expression patterns. Thus, given its potential as a master regulator of the intestinal phenotype, the induction and maintenance of ectopic Cdx1 and Cdx2 expression is likely to be a critical event in the development of Barrett's oesophagus.

Modelling Barrett's oesophagus using forward genetics

Forward genetics approaches have been extraordinarily helpful in advancing our understanding in a number of disease processes. Fundamentally, it involves the expression of a gene or combination of genes in order to elicit a disease phenotype. This approach was applied by Robert Weinberg's group as they defined the gene changes needed to transform normal cells into neoplastic ones [37]. To our knowledge, a forward genetics approach has not been widely applied to the problem of Barrett's oesophagus.

In one of the first published reports to adopt this approach, Cdx2 expression was induced in immortalized human oesophageal keratinocytes (HET1A cells). Cdx2 expression was associated with increased cell proliferation and an intestinal pattern of gene expression [38]. One important limitation of this study was that HET1A cells are immortalized by ectopic expression of the SV40 (simian virus 40) T-antigen, a viral oncoprotein which inactivates p53 and pRb (retinoblastoma protein), among other targets [39]. Inactivating p53 mutations are acquired late in Barrett's oesophagus [40]. Also, normal pRb function is critical for the antiproliferative effect of Cdx1 and possibly Cdx2 as well [41]. Thus the sequence of events leading to intestinalization may be significantly disordered when using HET1A cells as a cell culture model for Barrett's oesophagus.

We have subsequently reported on our studies using an immortalized, but not transformed, human oesophageal keratinocyte cell line called EPC2-hTERT [42,43]. These cells are immortalized by the ectopic expression of human telomerase. We used a retrovirus containing a Cdx2 cDNA and GFP (green fluorescent protein) reporter to infect the EPC2-hTERT cells in order to establish stable ectopic Cdx2 expression. In fact, we found it difficult to establish stable Cdx2 levels. Although we could immediately detect reporter GFP expression from the retrovirus infection, after 7–10 days in culture, a noticeable decline in GFP expression occurred only in those cells in which Cdx2 was co-expressed [44]. We demonstrated that Cdx2 expression significantly reduced EPC2-hTERT cell proliferation, which could only be rescued by increased cyclin D1 expression, but not a dominant-active p53 mutant. This was consistent with our work in colon cancer cells, where Cdx1 and Cdx2 have similar antiproliferative effects [18,24,45]. Together, these observations suggested that Cdx2 expression cannot be the first event in the transdifferentiation of oesophageal keratinocytes into Barrett's oesophagus epithelial cells. Some factor or factors driving cyclin D expression and cell proliferation would need to precede Cdx2 in order to sustain this cell population.

After establishing Cdx2 expression in our EPC2-hTERT.D1 cells, we evaluated the cells for evidence of intestinalization. We observed no significant changes in the morphology of EPC2-hTERT.D1.Cdx2 cells when compared with controls. We next explored for changes in gene-expression patterns by quantitative real-time PCR. We assayed a panel of genes associated with Barrett's oesophagus or Cdx2 expression in the intestine. In only one gene, *CA1* (carbonic anhydrase 1) did we observe a robust 7-fold increase in mRNA levels that was associated with Cdx2 expression. It suggests that Cdx2 expression alone is insufficient to induce intestinal transdifferentiation of normal oesophageal keratinocytes.

We then considered the possibility that epigenetic processes might be required for the intestinal transdifferentiation by Cdx2. Our EPC2-hTERT.D1.Cdx2 and control EPC2-hTERT.D1.MIGR1 cells were treated with 5-AzaC (5-aza-2-deoxycytidine), a DNA methyltransferase inhibitor. The 5-AzaC treatment enhanced the expression of several intestine-associated genes that were previously unexpressed [*DRA* (down-regulated in adenoma)/*SLC26A3* (solute carrier family 26, member A3), *LPH* (lactase phlorizin hydrolase), *KRT20* (keratin 20) and *CEACAM6* (carcinoembryonic antigen-related cell adhesion molecule 6)] or poorly expressed [*NHE2* (Na⁺-H⁺ exchanger 2)/*SLC9A2* (solute carrier family 9 member A2) and *VIL1* (villin 1)]. Some genes normally expressed in Barrett's oesophagus were not induced by the 5-AzaC treatment [*ALPI* (alkaline phosphatase, intestinal), *SI* (sucrase isomaltase) and *MUC2* (mucin 2)]. In other genes, there was clear evidence of a synergistic interaction between Cdx2 and 5-AzaC. mRNA levels for *CA1*, which were increased 7-fold with Cdx2 expression alone, and only 2-fold when treated with 5-AzaC,

increased by 35-fold when both treatments were applied. Most interesting of all, four genes responded only to the combination of Cdx2 expression and 5-AzaC treatment [*RETNLB* (resistin-like β), *NHE2*, *KRT20*, *DRA/SLC26A3*]. One of these genes, *KRT20*, is utilized as a marker for Barrett's oesophagus. Its induction is therefore of great importance. Two others (*NHE2* and *DRA/SLC26A3*) are highly expressed in intestinal epithelium, but were not previously known to be found in Barrett's oesophagus. We subsequently demonstrated that these two genes are frequently ectopically expressed in human Barrett's oesophagus. Taken together, these findings suggest that the intestinalization induced in EPC2-hTERT.D1-Cdx2 cells treated with 5-AzaC may mimic Barrett's oesophagus transdifferentiation, as two novel Barrett's oesophagus-associated markers were identified using this approach.

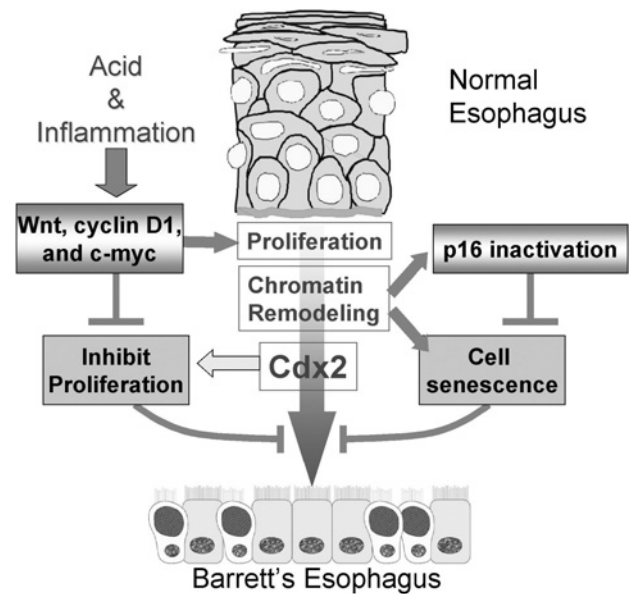
There was one important limitation to this approach. After 5-AzaC treatment, EPC2.hTERT.D1-Cdx2 and MIGR1 control cells exhibit altered cell morphology marked by increased cell volume and subconfluent growth arrest in the normal growth medium. These features are suggestive of cell senescence, which we confirmed by demonstrating induction of SA- β -gal (senescence-associated β -galactosidase) as well as the expression of p21^{Waf1/Cip1} and p16^{INK4a} after 5-AzaC treatment. The induction of cell senescence by 5-AzaC is a challenge for future studies using this reagent, but it does raise the possibility that senescence must be inactivated before the onset of the epigenetic changes required for Barrett's oesophagus.

In a final series of experiments, on the basis of our findings from the microarray analysis, we used c-Myc and Cdx1 co-expression to model Barrett's oesophagus in a novel three-dimensional culture system. EPC2-hTERT cells were engineered by retroviral infection to co-express c-Myc and Cdx1. c-Myc expression, like cyclin D1, appeared to be able to sustain EPC2-hTERT cell proliferation despite Cdx1 expression. Importantly, when these cells were placed in a three-dimensional organotypic culture system used previously to mimic the microenvironment of the oesophagus, a more significant intestinalization was observed [17]. A subpopulation of Alcian Blue-staining cells can be detected, and mucin 5AC expression was confirmed by immunohistochemistry. We conclude that the cellular microenvironment may provide additional cues to foster greater intestinalization when modelling Barrett's oesophagus *in vitro*.

Summary and future directions

These initial studies using a forward genetics approach to model Barrett's oesophagus have been highly informative despite their inability to induce fully intestinalized cells from an oesophageal keratinocyte precursor (Figure 2). We will need to build on these findings in future experiments. The literature from several intersecting fields may yield new insights and methodologies to better model Barrett's oesophagus *in vitro*. Elucidation of the mechanisms

Figure 2 | A model for future forward genetics approaches to modelling Barrett's oesophagus in cell culture systems



governing intestinal epithelial differentiation and cell-fate selection will be helpful. For instance, it has been determined that Wnt signalling in the intestine is essential for secretory cell fates [46]. Moreover, Wnt signalling during development can induce intestinal gene expression and morphology in lung endoderm, including the development of intestinal mucin-secreting goblet cells [47].

Other signalling pathways and transcription factors may also be implicated. The mATH1 (mouse atonal homologue 1)/hATH1 (human atonal homologue 1) transcription factor is required for the secretory cell lineage in the intestine, as loss of mATH1 leads to depletion of enteroendocrine, goblet and Paneth cells [48]. Notch, another developmentally critical signalling pathway, also regulates differentiation along cell lineages in the gut [49]. Notch signalling inhibits the selection of the secretory cell fate, in part through actions upon mATH1 expression [50]. The contribution of these signalling pathways, as well as others such as BMP4 (bone morphogenetic protein 4), has not yet been well studied in Barrett's oesophagus. However, given their role in intestinal development, it is likely that they perform a similar function in Barrett's oesophagus where the induction of the secretory cell fate, mucin-producing goblet cells, is observed.

In summary, the use of forward genetics approaches represents a powerful novel strategy to study and understand the pathophysiology of Barrett's oesophagus. Its application has already yielded new insights, including the timing of certain events, the role of pro-proliferation signals early in this process and the requirement for chromatin remodelling and epigenetic changes such that Cdx1 and Cdx2 can access their critical target genes (Figure 2). In the future, more complicated combinations of genes will be required, and the incorporation of three-dimensional culture techniques will

also be needed to promote greater adoption of the intestinal phenotype and cell morphology by keratinocytes.

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References

- Morales, C.P., Souza, R.F. and Spechler, S.J. (2002) Hallmarks of cancer progression in Barrett's oesophagus. *Lancet* **360**, 1587–1589
- Guillem, P.G. (2005) How to make a Barrett esophagus: pathophysiology of columnar metaplasia of the esophagus. *Dig. Dis. Sci.* **50**, 415–424
- Paulson, T.G. and Reid, B.J. (2004) Focus on Barrett's esophagus and esophageal adenocarcinoma. *Cancer Cell* **6**, 11–16
- Ouatu-Lascar, R., Fitzgerald, R.C. and Triadafilopoulos, G. (1999) Differentiation and proliferation in Barrett's esophagus and the effects of acid suppression. *Gastroenterology* **117**, 327–335
- Fitzgerald, R.C., Omary, M.B. and Triadafilopoulos, G. (1997) Acid modulation of HT29 cell growth and differentiation: an *in vitro* model for Barrett's esophagus. *J. Cell Sci.* **110**, 663–671
- Souza, R.F., Shewmake, K., Terada, L.S. and Spechler, S.J. (2002) Acid exposure activates the mitogen-activated protein kinase pathways in Barrett's esophagus. *Gastroenterology* **122**, 299–307
- Marchetti, M., Caliot, E. and Pringault, E. (2003) Chronic acid exposure leads to activation of the *cdx2* intestinal homeobox gene in a long-term culture of mouse esophageal keratinocytes. *J. Cell Sci.* **116**, 1429–1436
- Alvarez, H., Koorstra, J.B., Hong, S.M., Boonstra, J.J., Dinjens, W.N., Wu, T.T., Montgomery, E., Eshleman, J.R. and Maitra, A. (2008) Establishment and characterization of a *bona fide* Barrett esophagus-associated adenocarcinoma cell line. *Cancer Biol. Ther.* **7**, 1753–1755
- Palanca-Wessels, M.C., Klingelutz, A., Reid, B.J., Norwood, T.H., Opheim, K.E., Paulson, T.G., Feng, Z. and Rabinovitch, P.S. (2003) Extended lifespan of Barrett's esophagus epithelium transduced with the human telomerase catalytic subunit: a useful *in vitro* model. *Carcinogenesis* **24**, 1183–1190
- Jaiswal, K.R., Morales, C.P., Feagins, L.A., Gandia, K.G., Zhang, X., Zhang, H.Y., Hormi-Carver, K., Shen, Y., Elder, F., Ramirez, R.D. et al. (2007) Characterization of telomerase-immortalized, non-neoplastic, human Barrett's cell line (BAR-T). *Dis. Esophagus* **20**, 256–264
- Tobey, N.A., Hosseini, S.S., Argote, C.M., Dobrucali, A.M., Awayda, M.S. and Orlando, R.C. (2004) Dilated intercellular spaces and shunt permeability in nonerosive acid-damaged esophageal epithelium. *Am. J. Gastroenterol.* **99**, 13–22
- Jacobson, I., Poorkhalkali, N., Jonsson-Rylander, A.C. and Orlando, R.C. (2002) Effect of acid perfusion on passive electrophysiological properties of rabbit esophagus *in vivo*. *Dig. Dis. Sci.* **47**, 1369–1380
- Tatsuta, T., Mukaisho, K., Sugihara, H., Miwa, K., Tani, T. and Hattori, T. (2005) Expression of *Cdx2* in early GRCL of Barrett's esophagus induced in rats by duodenal reflux. *Dig. Dis. Sci.* **50**, 425–431
- Kumagai, H., Mukaisho, K., Sugihara, H., Bamba, M., Miyashita, T., Miwa, K. and Hattori, T. (2003) Cell kinetic study on histogenesis of Barrett's esophagus using rat reflux model. *Scand. J. Gastroenterol.* **38**, 687–692
- Nishijima, K., Miwa, K., Miyashita, T., Kinami, S., Ninomiya, I., Fushida, S., Fujimura, T. and Hattori, T. (2004) Impact of the biliary diversion procedure on carcinogenesis in Barrett's esophagus surgically induced by duodenoesophageal reflux in rats. *Ann. Surg.* **240**, 57–67
- Theisen, J., Peters, J.H., Fein, M., Hughes, M., Hagen, J.A., Demeester, S.R., Demeester, T.R. and Laird, P.W. (2005) The mutagenic potential of duodenoesophageal reflux. *Ann. Surg.* **241**, 63–68
- Stairs, D.B., Nakagawa, H., Klein-Szanto, A., Mitchell, S.D., Silberg, D.G., Tobias, J.W., Lynch, J.P. and Rustgi, A.K. (2008) *Cdx1* and *c-Myc* foster the initiation of transdifferentiation of the normal esophageal squamous epithelium toward Barrett's esophagus. *PLoS ONE* **3**, e3534
- Guo, R.J., Suh, E.R. and Lynch, J.P. (2004) The role of *Cdx* proteins in intestinal development and cancer. *Cancer Biol. Ther.* **3**, 593–601
- Traber, P.G., Wu, G.D. and Wang, W. (1992) Novel DNA-binding proteins regulate intestine-specific transcription of the sucrase-isomaltase gene. *Mol. Cell. Biol.* **12**, 3614–3627
- Suh, E., Chen, L., Taylor, J. and Traber, P.G. (1994) A homeodomain protein related to *caudal* regulates intestine-specific gene transcription. *Mol. Cell. Biol.* **14**, 7340–7351
- Dang, D.T., Mahatan, C.S., Dang, L.H., Agboola, I.A. and Yang, V.W. (2001) Expression of the gut-enriched Krüppel-like factor (Krüppel-like factor 4) gene in the human colon cancer cell line RKO is dependent on *CDX2*. *Oncogene* **20**, 4884–4890
- Funakoshi, S., Ezaki, T., Kong, J., Guo, R.J. and Lynch, J.P. (2008) Repression of the desmocollin 2 gene in colorectal cancer cells is relieved by the homeodomain transcription factors *Cdx1* and *Cdx2*. *Mol. Cancer Res.* **6**, 1478–1490
- Lynch, J., Suh, E.R., Silberg, D.G., Rulyak, S., Blanchard, N. and Traber, P.G. (2000) The *caudal*-related homeodomain protein *Cdx1* inhibits proliferation of intestinal epithelial cells by down-regulation of D-type cyclins. *J. Biol. Chem.* **275**, 4499–4506
- Lynch, J., Keller, M., Guo, R., Yang, D. and Traber, P.G. (2003) *Cdx1* inhibits the proliferation of human colon cancer cells by reducing cyclin D1 gene expression. *Oncogene* **22**, 6395–6407
- Keller, M.S., Ezaki, T., Guo, R.J. and Lynch, J.P. (2004) *Cdx1* or *Cdx2* expression activates E-cadherin-mediated cell-cell adhesion and compaction in human Colo 205 cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **287**, G104–G114
- Ezaki, T., Guo, R.J., Li, H., Reynolds, A.B. and Lynch, J.P. (2007) The homeodomain transcription factors *Cdx1* and *Cdx2* induce E-cadherin adhesion activity by reducing β - and p120-catenin tyrosine phosphorylation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **293**, G54–G65
- Suh, E. and Traber, P.G. (1996) An intestine-specific homeobox gene regulates proliferation and differentiation. *Mol. Cell. Biol.* **16**, 619–625
- Gao, N., White, P. and Kaestner, K.H. (2009) Establishment of intestinal identity and epithelial-mesenchymal signaling by *Cdx2*. *Dev. Cell* **16**, 588–599
- Bai, Y.Q., Yamamoto, H., Akiyama, Y., Tanaka, H., Takizawa, T., Koike, M., Kenji Yagi, O., Saitoh, K., Takeshita, K., Iwai, T. and Yuasa, Y. (2002) Ectopic expression of homeodomain protein *CDX2* in intestinal metaplasia and carcinomas of the stomach. *Cancer Lett.* **176**, 47–55
- Silberg, D.G., Furth, E.E., Taylor, J.K., Schuck, T., Chiou, T. and Traber, P.G. (1997) *CDX1* protein expression in normal, metaplastic, and neoplastic human alimentary tract epithelium. *Gastroenterology* **113**, 478–486
- Satoh, K., Mutoh, H., Eda, A., Yanaka, I., Osawa, H., Honda, S., Kawata, H., Kihira, K. and Sugano, K. (2002) Aberrant expression of *CDX2* in the gastric mucosa with and without intestinal metaplasia: effect of eradication of *Helicobacter pylori*. *Helicobacter* **7**, 192–198
- Phillips, R.W., Frierson, Jr, H.F. and Moskaluk, C.A. (2003) *Cdx2* as a marker of epithelial intestinal differentiation in the esophagus. *Am. J. Surg. Pathol.* **27**, 1442–1447
- 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
- Eda, A., Osawa, H., Yanaka, I., Satoh, K., Mutoh, H., Kihira, K. and Sugano, K. (2002) Expression of homeobox gene *CDX2* precedes that of *CDX1* during the progression of intestinal metaplasia. *J. Gastroenterol.* **37**, 94–100
- Silberg, D.G., Sullivan, J., Kang, E., Swain, G.P., Moffett, J., Sund, N.J., Sackett, S.D. and Kaestner, K.H. (2002) *Cdx2* ectopic expression induces gastric intestinal metaplasia in transgenic mice. *Gastroenterology* **122**, 689–696
- 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
- Hahn, W.C., Counter, C.M., Lundberg, A.S., Beijersbergen, R.L., Brooks, M.W. and Weinberg, R.A. (1999) Creation of human tumour cells with defined genetic elements. *Nature* **400**, 464–468
- Liu, T., Zhang, X., So, C.K., Wang, S., Wang, P., Yan, L., Myers, R., Chen, Z., Patterson, A.P., Yang, C.S. and Chen, X. (2006) Regulation of *Cdx2* expression by promoter methylation, and effects of *Cdx2* transfection on morphology and gene expression of human esophageal epithelial cells. *Carcinogenesis* **28**, 488–496

- 39 Ahuja, D., Saenz-Robles, M.T. and Pipas, J.M. (2005) SV40 large T antigen targets multiple cellular pathways to elicit cellular transformation. *Oncogene* **24**, 7729–7745
- 40 Tselepis, C., Perry, I. and Jankowski, J. (2000) Barrett's esophagus: dysregulation of cell cycling and intercellular adhesion in the metaplasia–dysplasia–carcinoma sequence. *Digestion* **61**, 1–5
- 41 Haigis, K., Sage, J., Glickman, J., Shafer, S. and Jacks, T. (2006) The related retinoblastoma (pRb) and p130 proteins cooperate to regulate homeostasis in the intestinal epithelium. *J. Biol. Chem.* **281**, 638–647
- 42 Harada, H., Nakagawa, H., Oyama, K., Takaoka, M., Andl, C.D., Jacobmeier, B., von Werder, A., Enders, G.H., Oritz, O.G. and Rustgi, A.K. (2003) Telomerase induces immortalization of human esophageal keratinocytes without p16INK4a inactivation. *Mol. Cancer Res.* **1**, 729–738
- 43 Okawa, T., Michaylira, C.Z., Kalabis, J., Stairs, D.B., Nakagawa, H., Andl, C.D., Johnstone, C.N., Klein-Szanto, A.J., El-Deiry, W.S., Cukierman, E. et al. (2007) The functional interplay between EGFR overexpression, hTERT activation, and p53 mutation in esophageal epithelial cells with activation of stromal fibroblasts induces tumor development, invasion, and differentiation. *Genes Dev.* **21**, 2788–2803
- 44 Kong, J., Nakagawa, H., Isariyawongse, B., Funakoshi, S., Silberg, D., Rustgi, A.K. and Lynch, J.P. (2008) Induction of intestinalization in human esophageal keratinocytes is a multistep process. *Carcinogenesis* **30**, 122–130
- 45 Guo, R.J., Huang, E., Ezaki, T., Patel, N., Sinclair, K., Wu, J., Klein, P.S., Suh, E. and Lynch, J.P. (2004) Cdx1 inhibits human colon cancer cell proliferation by reducing β -catenin/TCF transcriptional activity. *J. Biol. Chem.* **279**, 36865–36875
- 46 van Es, J.H., Jay, P., Gregorieff, A., van Gijn, M.E., Jonkheer, S., Hatzis, P., Thiele, A., van den Born, M., Begthel, H., Brabletz, T. et al. (2005) Wnt signalling induces maturation of Paneth cells in intestinal crypts. *Nat. Cell Biol.* **7**, 381–386
- 47 Okubo, T. and Hogan, B.L. (2004) Hyperactive Wnt signaling changes the developmental potential of embryonic lung endoderm. *J. Biol.* **3**, 11
- 48 Yang, Q., Bermingham, N.A., Finegold, M.J. and Zoghbi, H.Y. (2001) Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. *Science* **294**, 2155–2158
- 49 Tepass, U., Godt, D. and Winklbauer, R. (2002) Cell sorting in animal development: signalling and adhesive mechanisms in the formation of tissue boundaries. *Curr. Opin. Genet. Dev.* **12**, 572–582
- 50 Fre, S., Huyghe, M., Mourikis, P., Robine, S., Louvard, D. and Artavanis-Tsakonas, S. (2005) Notch signals control the fate of immature progenitor cells in the intestine. *Nature* **435**, 964–968

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