Barrett’s metaplasia: molecular mechanisms and nutritional influences

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Abstract

Barrett’s metaplasia is discussed in the context of a general theory for the formation of metaplasias based on developmental biology. The phenotype of a particular tissue type becomes established during embryonic development by the expression of a specific set of transcription factors. If this combination becomes altered, then the tissue type can be altered. Such events may occur by mutation or by environmental effects on gene expression, normally within the stem cell population of the tissue. A macroscopic patch of metaplastic tissue will arise only if the new gene activity state is self-sustaining in the absence of its original causes, and if the new tissue type can outgrow the parent tissue type. An important candidate gene for the causation of Barrett’s metaplasia is Cdx2 (Caudal-type homeobox 2). In normal development, this is expressed in the future intestine, but not the future foregut. Mouse knockout studies have shown that it is needed for intestinal development, and that its loss from adult intestine can lead to squamous transformations. Overexpression of Cdx2 in oesophageal cell cultures by treatment with bile acids. We have investigated the ability of Cdx2 to bring about intestinal transformations in oesophageal epithelium. Our results show that Cdx2 can activate a programme of intestinal gene expression when overexpressed in HET-1A cells, or in fetal epithelium, but not in the adult epithelium. This suggests that Cdx2, although necessary for formation of intestinal tissue, is not sufficient to provoke Barrett’s metaplasia in adult life and that overexpression of additional transcription factors is necessary. In terms of diet and nutrition, there is a known association of Barrett’s metaplasia with obesity. This may work through an increased risk of gastro-oesophageal reflux. Acid and bile are known to activate Cdx2 expression in oesophageal cells. It may also increase circulating levels of TNFα (tumour necrosis factor α), which activates Cdx2. In addition, there may be effects of diet on the composition of the bile.

Introduction

Barrett’s metaplasia involves a replacement of the normal stratified squamous epithelium of the distal oesophagus by columnar epithelium, which contains cell types similar to the normal intestinal epithelium [1,2]. It is usually found in the context of GORD (gastro-oesophageal reflux disease) and is thought to arise as a consequence of the damage to the epithelium provoked by acid and bile [3,4]. Apart from posing an interesting problem for developmental biologists, Barrett’s metaplasia is of clinical importance because it is the only known precursor to OAC (oesophageal adenocarcinoma), which has increased dramatically in the Western world over the last 30 years, at a higher rate than any other cancer [5,6].

The concept of metaplasia carries with it three basic ideas. First, that a region of tissue is located in the wrong place; secondly, that it appeared there in postnatal life rather than in embryonic development; and thirdly, that it actually developed in the wrong place rather than migrating there from elsewhere. Ectopic tissue arising during embryonic...
Figure 1 A simple developmental hierarchy

Each variation of tissue-type switching (transdifferentiation (1), metaplasia (2), dysplasia (3) and transformation to induced pluripotent stem cell (4)) represents an abnormal transition compared with normal development. Reproduced from [58] with permission.

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development is usually referred to as a heterotopia rather than a metaplasia, although there may be some overlap of mechanism involved in their formation. Tissues misplaced because of an abnormal migration during embryogenesis [7] represent a quite different type of mechanism. Evidence that Barrett’s metaplasia is a genuine metaplasia comes from three sources. First, it is much more common in adults than in children [8]; secondly, the presence of intestinal tissue is unlikely to be due to overgrowth; and thirdly, animal experiments have shown that Barrett’s metaplasia can be induced even when there is a gap between the oesophageal epithelium and the oesophageal–gastric junction [9].

Theory about how metaplasia arises

The process of regional specification in embryonic development is now quite well understood [10]. It proceeds in a hierarchical manner. Starting from the epiblast of the early embryo, each tissue rudiment is formed by a sequence of developmental decisions. At each step, a particular combination of transcription factors is activated or repressed in response to a particular extracellular signal, which may be composed of one or more inducing factors (Figure 1). Different concentrations of the signal or transcription factors will result in the adoption of a different developmental pathway. Hence, each step leads to multiple pathways, a developmental ‘choice’.

We know that it is not necessary to change the activity of hundreds of genes to alter a cell phenotype, because development is controlled by a relatively small number of genes encoding those transcription factors whose activity determines developmental choices between programmes of gene expression. These critical genes are sometimes called ‘master control genes’, and the misexpression of these genes is the key to understanding metaplasia.

Some years ago, I proposed a unified theory for metaplasias based on the misexpression of developmental control genes [11–13]. There are five key elements to this theory.

(i) Different body parts arise in embryonic development because different, specific, combinations of genes encoding transcription factors become activated. Such a combination constitutes a ‘code’ specifying the body part in question. The code is built up through a hierarchy of developmental decisions, mostly depending on the response of cells to inducing factors secreted by neighbouring tissues. The code leads, directly or indirectly, to the activation of the relevant set of differentiation genes.

(ii) Many body parts are associated with specific tissue types, for example each of the organs derived from the endoderm of the early embryo, has its own specific epithelium (e.g. oesophageal, gastric, intestinal or hepatic).

(iii) Wherever a tissue is sustained by cell turnover, there are stem cells that persist through adult life. It is assumed that these cells retain the same codes as the embryonic primordia.

(iv) Whenever the code is changed, whether by mutation, epigenetic switching or environmental effects such as hormone action, then the tissue type produced by those stem cells will change. A metaplasia will arise if the code is changed to another normal code and other changes, such as dysplasias, may arise when abnormal codes are generated. The initial change need only occur in only one or a few cells. If the new tissue type has a growth advantage over the old, it can expand to become a macroscopic focus of metaplasia.

(v) The probability of metaplasia increases with regeneration. This is because tissue damage means that stem cell niches need to be repopulated with new cells, giving the opportunity for a metaplastic focus to expand to visible size.

In the present paper, I shall consider how this model applies to the oesophagus and to the formation of Barrett’s metaplasia.

Oesophageal development

The oesophageal epithelium is formed from the foregut region of the endoderm [14], and several transcription factors are probably involved in its determination, a few of which are shown in Figure 2. As the gut tube is forming, the Caudal-related factor Cdx2 (Caudal-type homeobox 2) is expressed in the whole prospective intestine, and the SRY (sex-determining region Y)-box factor Sox2 in the prospective oesophagus and stomach, later fading from the stomach. These expression domains probably depend on inductive signals from the gut mesenchyme, which can activate both Sox2 and Cdx2 genes in heterotopic combinations [15]. In loss-of-function mutants of Cdx2, the intestinal lining becomes transformed into a squamous epithelium resembling the oesophagus [16]. Mice heterozygous for Cdx2 sometimes lose expression of the active allele and develop foci of stratified squamous epithelium within areas of colon and small intestine.
Expression domains of transcription factors in the endodermal layer of the early chick gut.

Sox2

cdx2

Cdx2

Figure 2 | Expression domains of transcription factors in the endodermal layer of the early chick gut.

Developmental progression occurred by transdifferentiation of the epithelium or by overgrowth from the neighbouring oral epithelium, which is normally stratified. We investigated this using organ cultures from fetal mouse oesophagus [27]. From three separate lines of evidence, we concluded that transdifferentiation did occur. First, a few cells can be detected in the basal layer which share expression of the columnar marker cytokeratin 8 (K8) and the squamous marker cytokeratin 14 (K14). Secondly, if a reporter is introduced to the columnar K8-positive cells, driven by the K14 promoter, this will become activated. Thirdly, the process of epithelial morphology change was unaffected by inhibitors of cell division or cell death, showing that selective overgrowth cannot be responsible. The fact that a primitive columnar–squamous transition takes place in normal development is of obvious significance in terms of the apparent change in Barrett’s metaplasia from squamous to columnar, as it suggests an element of reversal of the normal developmental progression.

Role of Cdx2 in Barrett’s metaplasia

There is an impressive accumulation of evidence that the Cdx genes are involved in the initiation of Barrett’s metaplasia [28]. Both Cdx1 and Cdx2 are expressed in Barrett’s metaplasia tissue [29–31]. In transgenic mouse studies in which these genes are ectopically expressed in embryonic stages, either gene is sufficient to establish intestinal tissue within the gastric epithelium [32–34]. Acid and bile, the toxic ingredients of gastric reflux to the oesophagus, have been shown to induce expression of Cdx1 and Cdx2 in oesophageal cells [31,35]. Investigations of intestinal metaplasia in the biliary system also provided suggestive data. In biliary epithelial cells, the three cytokines TNFα (tumour necrosis factor α), IL (interleukin)-1β and IL-6, induce Cdx2 expression via a NF-κB (nuclear factor κB)-dependent mechanism [36,37]. This observation provides a mechanistic link between inflammation and ectopic Cdx2 expression. This collected evidence makes up a strong case for Barrett’s metaplasia being due to an ectopic activation of Cdx2 in the oesophagus in adult life.

In order to make a direct test of the ability of ectopic Cdx2 to activate an intestinal programme of gene expression in the oesophagus, we have utilized in vitro organ cultures of fetal and adult oesophagus. Unlike tissue culture of cell monolayers, organ cultures have the advantage of preserving the normal tissue organization, and may combine this with the ability to introduce genes for overexpression studies. It is never easy to introduce genes to the epithelial component of organ cultures, but we have had success using adenovirus, especially when the cultures were treated with dispase to slightly separate the epithelial cells [38]. We believe that this treatment makes accessible the CAR (coxsackie virus and adenovirus receptor) found associated with tight junctions.

The results indicate that the fetal oesophagus does activate intestinal gene products in response, but the adult oesophagus does not. There are some effects on the adult oesophagus,
including a reduction of p63 expression in some cells expressing Cdx2. As mentioned above, p63 has many of the characteristics of a ‘master gene’ controlling squamous differentiation so suppression of its activity is a necessary step in the squamous to columnar transition. But evidently, the loss of p63 is not enough to activate an intestinal gene expression programme in this system.

Cdx1 and/or Cdx2 are generally believed to be sufficient for formation of intestinal tissue in the stomach, on the basis of transgenic overexpression experiments using the stomach-specific Na⁺/K⁺-ATPase promoter [32,39,40]. But this promoter activates Cdx2 expression quite early in fetal life, so the result can be compared with our overexpression in fetal organ cultures. Other studies in which Cdx overexpression activates intestinal markers have been in tissue culture cells, such as the HET-1A cells, where the phenotype is somewhat divergent from normal oesophageal epithelium. Moreover, studies which involve microarray profiling of cells overexpressing Cdx2 show only rather modest increases in expression of the intestinal target genes, which are unlikely to be sufficient to change the cell morphology to an intestinal type [41,42].

Cdx2 is certainly necessary for the normal development of the intestine, and for distinguishing intestinal- and oesophageal-type epithelia. However, on the basis of our experiments, we conclude that it is probably not sufficient on its own to provoke the onset of Barrett’s metaplasia in adult tissue. Probably, it is active in fetal tissue because this is still somewhat labile, with the closure of genetic targets by chromatin modifications and DNA methylation not yet being complete. A similar situation may exist in tissue culture cells such as HET-1A which have properties intermediate between the squamous and the columnar.

Barrett’s metaplasia and its nutritional associations

The epidemiological literature on Barrett’s metaplasia and oesophageal adenocarcinoma is complex. In general, there is a well-established association of Barrett’s metaplasia with obesity. However, there is dispute about whether this is entirely attributable to a greater risk of GORD [43,44], or whether there are independent effects of obesity. It is probable that the risk of carcinoma is not increased further by obesity once the metaplasia is established [45]. There is also a probable negative association with Helicobacter pylori infection in the stomach [8]. The importance of understanding the molecular mechanisms of pathological processes is that we can start to make sense of data arrived at by epidemiological methods, and to decide what is directly causal and what is merely an association with some unknown third factor.

Although activation of Cdx2 may not be sufficient to initiate Barrett’s metaplasia, it is almost certainly a necessary event. GORD involves the reflux of stomach acid and some bile acids into the distal oesophagus. Although the normal concentration of bile acids in the distal oesophagus is close to zero, patients with Barrett’s metaplasia have measured concentrations of approx. 180 μM [46]. Several studies have shown that these agents can activate expression of Cdx2 and other intestinal genes in oesophageal cell cultures [35,47,48]. The cells in these studies include primary cultures of oesophageal epithelium as well as the less reliable tissue culture models. The intracellular signalling pathway for gene activation is the NK-κB pathway, often activated under conditions of stress or inflammation.

What is the role of obesity? Obesity increases the risk of GORD for several reasons: the obese are more likely to have a hiatus hernia, intra-abdominal pressure is higher and there is likely to be more pancreatic enzymes and bile in the refluxate [8]. But there are other possibilities as well. One concerns the cytokine TNFα. This is synthesized by adipose tissue and the blood levels are higher in obese individuals [49,50]. TNFα has also been shown to activate the NF-κB pathway in oesophageal cells, leading to the expression of Cdx2 [47].

The central importance of the effect of bile acids focuses attention on the level and composition of bile in the stomach. Experiments with oesophageal cells show that cholic acid and the related dehydrocholic acid show more activity than
all the other bile acids [31]. Bile acids are formed in the liver from cholesterol as cholic and chenodeoxycholic acids. These become conjugated with glycine or taurine and enter the intestine with the bile. In the intestine, they become deconjugated and dehydroxylated by bacteria, leading to a conversion of cholic into deoxycholic acid. A high proportion of the bile acids in the intestine are reabsorbed in the ileum and are then recirculated back to the bile. There are documented changes of concentrations and proportions of the different bile acids in the presence of a high-fat diet [51]. So an altered dietary state might generate a change of intestinal flora and a reduction of bacterial formation of deoxycholic acid and retention of a higher steady-state level of cholic acid in the intestine. When this enters the oesophagus as a result of GORD, it will have a stronger Barrett’s metaplasia-inducing effect.

One of the explanations for the increase in Barrett’s metaplasia in recent decades is the reduction in the prevalence of H. pylori infection, owing to better hygiene and nutrition. The mechanism of this effect can only be speculated on, but it has been shown that H. pylori in the stomach correlates negatively with the presence of bile acids >1 mM [52], so this effect too may be via the effects of bile acids on the oesophageal lining. Alternatively, or in addition, the cagA+ variant, most associated with the protective effect, does seem to reduce gastric acidity after infection, making the effects of GORD less severe [53].

This discussion only comprises suggestions about the possible effects of diet, obesity and H. pylori on the formation of Barrett’s metaplasia. The evidence is circumstantial, and many of the molecular studies are carried out on oesophageal tumour cell lines that may diverge substantially from phenotype from the normal epithelium. However, the more that we learn about the molecular pathogenesis, the more it will become clear how the effects of diet and nutrition are mediated.

Causes and progression
Barrett’s metaplasia is normally considered to arise in adult life, because there is a higher prevalence in adults than children, and because it is believed to be caused by GORD. However, macroscopic lesions generally commence as microscopic ones. In view of the susceptibility of the fetal oesophagus to Cdx2 overexpression, it is also possible that the initial stage of Barrett’s metaplasia is really a developmental heterotopia. Such lesions could be very small, just one or a few stem cells plus their clonal progeny, which would not be visible as a macroscopic lesion by endoscopy. Then, in adult life, the effects of GORD would be to destroy the surrounding squamous epithelium. If there is just a small growth advantage of the Barrett’s metaplasia tissue under these conditions, then it will eventually spread out and occupy more space. This possibility does not depend on whether the cell or origin is actually a basal layer cell in the main epithelium, or a cell in one of the oesophageal glands [54]. One piece of evidence for an early initiating event is the age cohort effect seen by El Serag et al. [55], which indicates that conditions during development or early childhood may affect the later frequency of Barrett’s metaplasia.

In the cancer field, much attention has been devoted to the progression from Barrett’s metaplasia through grades of dysplasia to oesophageal adenocarcinoma [36]. As in other malignancies, this progression involves a whole variety of events, including epigenetic changes, somatic mutations and chromosome alterations. Recent studies on the clonal composition of Barrett’s metaplasia using laser-microdissected regions of homogeneous appearance indicates the presence of a complex mixture of clones with different somatic mutations, some representing dysplastic area that may progress to carcinoma [57]. But the complex composition of Barrett’s metaplasia also exists before the dysplasia stage. Much of the difficulty of defining the condition precisely is that it involves both a general columnar phenotype and an intestine-like one. Both from the biological standpoint of understanding the mechanism and from the clinical standpoint of preventing or curing disease, we need to know a lot more about the actual sequence of events in the metaplasia–dysplasia–cancer sequence, starting with the initiating event that might even be so inscrutable as to lie within a single cell during fetal development.

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