Turning Skyscrapers into Town Houses: Insights into Barrett's Esophagus

Theresa D. Ahrens^{a, b} Lisa Lutz^a Silke Lassmann^{a, c–e} Martin Werner^{a, d, e}

^aInstitute for Surgical Pathology, Medical Center and Faculty of Medicine, ^bFaculty of Biology and ^cBIOSS Centre for Biological Signalling Studies, University of Freiburg, and ^dComprehensive Cancer Center Freiburg, Freiburg, and

^eGerman Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany

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Abstract

Barrett's esophagus (BE) is defined as metaplasia of the esophageal squamous epithelium with multiple cell layers into a single layer of intestinal columnar epithelial cells – or, in other words, skyscrapers are turned into town houses. The underlying pathomechanism(s) and the cell of origin of BE lesions have not been defined yet. However, four potential hypotheses for BE development have been suggested. The morphological changes during BE development are associated with rather well-described aberrant gene/protein expression patterns. However, the potential key regulators of this conversion process are still unclear. The process of metaplastic conversion is difficult to monitor in a spatiotemporal manner in vitro, and robust models are lacking. There is therefore a need for novel experimental systems. This review focuses on potential key regulators, microenvironmental influences, epigenetic alterations and experimental research systems related to BE.

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Introduction

Barrett's esophagus (BE) develops via replacement of the esophageal squamous epithelium with an intestinal columnar epithelium, representing a metaplastic lesion (fig. 1a). This describes several major changes in histological architecture in just a few words. An analogy may help the understanding of the fundamental conversion: the normal esophageal epithelium is a stratified squamous epithelium with multiple cell layers, comparable to 'skyscrapers' with different floors. In contrast, the epithelium of BE consists of a unilayered, columnar epithelium or 'at-grade town houses' (fig. 1b). During BE development, a great departure from the biological construction plan occurs, with skyscrapers being turned into town houses. BE lesions may then proceed via dysplastic changes to Barrett's adenocarcinomas (BACs; fig. 1a).

Histological evidence of goblet cells in endoscopic biopsies is essential for BE diagnosis in most countries, although the classification of BE is different in Japan and Great Britain [1, 2], where the presence of metaplastic columnar epithelium is sufficient for the diagnosis of BE.

Fig. 1. **a** BE develops via conversion of the normal, squamous esophageal epithelium (NE) into an intestinal columnar epithelium. Some pathology societies demand the presence of goblet cells for BE diagnosis (see Introduction). BE lesions may proceed via dysplastic states, also called intraepithelial neoplasia (IEN), with frequent loss of goblet cells, to BAC. **b** These fundamental changes

in histological architecture are possibly more understandable via an analogy: skyscrapers represent the normal esophageal squamous epithelium with its different layers, and these are then turned into little town houses via unknown mechanisms, representing the BE lesions with columnar epithelium.

Heterotopic columnar epithelium of cardia or fundus type, which is originally in the direct neighborhood of the esophagogastric junction, lacks goblet cells and may, according to the guidelines in Japan and Great Britain, be misinterpreted as BE.

Despite the set histological classifications for BE lesions and their grade of dysplasia [2, 3], there is a remarkable interobserver variability among pathologists [2, 4]. According to the WHO, every BE lesion should be classified into one of the following 4 categories: negative for dysplasia, indefinite for dysplasia, positive for low-grade dysplasia and positive for high-grade dysplasia [5, 6]. The interobserver variability is most prevalent for the differentiation between low-grade dysplasia versus indefinite for dysplasia [4]. Here, the elucidation of the underlying molecular pathomechanisms may be a valuable approach to more precisely define BE lesions and to improve the reproducibility of the histological evaluation, especially of the dysplasia classification. For example, characteristic expression patterns of key pathomechanistic proteins could facilitate BE diagnostics and subclassification, thereby underscoring/supplementing the histological

detection of (low- or high-grade) dysplasia in BE lesions, which still remains the best biomarker for the risk stratification of a risk of progression to BAC [1] and hence prognosis.

The underlying molecular pathomechanisms of BE development are not well elucidated. However, BAC as a potential malignant end point of BE shows a dramatic increase in Western countries [7]. Actually, the occurrence of BE lesions is one major risk factor for developing BAC, with recently reported annual conversion rates of between 0.01 and 0.22%, depending mainly on the length of BE [8]. These annual conversion rates are clearly lower than the estimates reported 5 years ago [9]. Actually, esophageal adenocarcinomas (EACs) are a heterogeneous group of tumors, which are often localized close to the esophagogastric junction [10, 11].

This review focuses on recent advances in defining the cellular origin of BE lesions as well as the mechanisms involved, including changes in protein expression and epigenetic aspects. In addition, potential systems for BE 'bench-side' research will be discussed.

Four Potential Molecular Routes towards BE

Two hypotheses for the cellular origin of BE lesions are currently in focus: ‘true’ trans-differentiation of the normal esophageal epithelium (route 1) or wrong differentiation of esophageal stem cells residing in the basal cell layers or in the esophageal glands, also termed ‘trans-commitment’ (route 2) [12, 13].

Embryonic development shows that the human esophagus develops from a columnar epithelium which is replaced from the middle by rostral and caudal extension of a stratified squamous epithelium [14, 15]. Yu et al. [16] revealed that esophageal columnar/keratin-8⁺ cells differentiate via a double-positive state into squamous/keratin-14⁺ cells. This demonstrates the capacity of esophageal epithelial cells to undergo ‘true’ trans-differentiation, thus clearly supporting the trans-differentiation model (route 1). This theory was further strengthened by Mari et al. [17], who recently excluded the involvement of Lgr5⁺ progenitor cells from neighboring columnar intestinal epithelia in the formation of esophageal metaplasia.

The trans-commitment of esophageal stem cells with wrong differentiation (route 2) has not been clearly proven so far and even these stem cells themselves have not been finally defined [18]. But recent work in mice revealed that the esophageal epithelium is maintained by a distinct progenitor population [19]. The link of BE development and esophageal stem cells was recently reviewed by Barbera and Fitzgerald [20].

Furthermore, colonization by bone marrow stem cells with subsequent trans-commitment (route 3) for the BE development is under discussion [21, 22]. In a study by Sarosi et al. [21] in 2008, female rats were lethally irradiated and transplanted with bone marrow from male rats. This allowed the use of the Y-chromosome as a marker for bone marrow cell colonization in organs. Female rats with reflux esophagitis caused by esophagojejunostomy showed areas of intestinal metaplasia. Indeed, half of the squamous epithelial cells (49.6%) stained positive for Y-chromosome by FISH, indicating the descent from the transplanted male bone marrow cells. Furthermore, the authors excluded the possibilities of the fusion of bone marrow cells and epithelial cells or invading white blood cells. This theory was further strengthened by Hutchinson et al. [22] in 2011 with a mouse model. They also detected the occurrence of cells with female karyotype within the BAC tumor in one male patient after bone marrow transplantation. This provides at least an *in vivo* proof-of-principle for the involvement of bone marrow-derived

cells in BE lesions, but it is still questionable whether these cells are causative in the development of BE.

The fourth route to BE lesions is cellular competition between esophageal squamous cells and a foreign cell lineage. This older concept suggests that BE lesions may arise by replacement with columnar cells of gastric or junctional origin [23]. Indeed, a recent study on human esophageal tissues after IdU labelling revealed that cells expressing Lgr5⁺ Ki-67⁺ residing in the middle of BE glands showed a bidirectional flux of cells towards the surface and into the base of the BE glands, reflecting the gland architecture of antral or pyloric mucosa of the stomach [24]. This finding points towards BE originating from the gastric epithelium. Quante et al. [25] also suggested that Lgr5⁺ cells within the cardiac mucosa are responsible for BE formation. Moreover, McDonald et al. [26] have recently suggested that BE lesions derive from cardiac stem cells by revisiting the morphological architecture of human Barrett mucosa. Further, cellular competition could also be mediated by embryonic residual cells. A study on a murine p63-null model suggests that residual Car4⁺ embryonic cells give rise to metaplastic esophageal epithelium by cellular competition, independent of a trans-commitment mechanism [27].

In summary, there are currently 4 potential routes for the development of BE (fig. 2): (1) trans-differentiation of the esophageal epithelium, (2) trans-commitment by wrong differentiation of stem cells either of the esophageal epithelium itself or of the submucosal glands, (3) colonization by bone marrow-derived stem cells and (4) cellular competition, e.g. by the upward progression of cardiac stem cells. Routes (2) and (3) may represent ‘similar’ mechanisms, with different origins of the stem cells involved, i.e. from the esophageal niche itself or from the bone marrow, but both these stem cell populations show a dramatic switch in their differentiation programs. Indeed, these 4 routes are maybe not mutually exclusive and BE pathogenesis may also vary, depending on the background of the individual patient.

Expression Pattern Changes Associated with BE Lesions

Not surprisingly, the morphologic conversion of squamous epithelial cells into intestinal columnar epithelial cells during BE development is reflected by changes in gene and protein expression patterns (fig. 3, summary).

This refers especially to proteins associated with cellular differentiation or cell-to-cell interactions. For exam-

Fig. 2. Potential routes to the development of BE. The exact mechanisms by which the normal esophageal squamous epithelium is turned into BE have not been well elucidated. However, 4 potential development routes have been described so far: the trans-differen-

tiation (1) and trans-commitment of stem cells of either esophageal (2) or bone marrow origin (3) and cellular competition (4). Each of this hypotheses may explain the development of BE lesions and may depend on the background of the individual patient.

Fig. 3. Changes during the development of BE. The exact events initiating the development of BE are still largely unknown. However, changes in the microenvironment such as increased acid levels due to reflux or chemokine expression are known to initiate BE lesions. Moreover, key transcription factors like CDX-2 are up-

regulated, which then mediates complex changes in the epigenetic landscape including promoter demethylation or miRNA alterations. This leads, finally, to gene/protein expression changes associated with differentiation programs and the subsequent upregulation of columnar markers (e.g. keratin-7).

ple, BE lesions show a switch of cytokeratin expression from squamous (e.g. keratin-5/6 or keratin-14) towards columnar markers like keratin-7, keratin-8 or keratin-18 [28]. Moreover, claudin-3 and claudin-4 are specifically upregulated in BE lesions compared to normal esophageal epithelia or esophageal squamous cell carcinoma (ESCC) [29]. Isoform 2 of claudin-18 is also upregulated in BE and may foster resistance to acidic conditions [30].

Mucin expression changes during BE development. Normal esophagus shows MUC1 and MUC4 expression in squamous epithelial cells as well as MUC5B expression in the esophageal glands [31]. In contrast, BE lesions show upregulation of the gastric mucins MUC5A and MUC6 [31, 32]. Here, MUC5A is expressed in the surface epithelium and MUC6 expression is localized to the deeper glands, resembling an expression pattern seen in the gastric mucosa [33].

MUC2 is expressed specifically in goblet cells [34, 35]. Indeed, MUC2 is directly linked to goblet cell differentiation since *Muc2*^{-/-} mice show a lack of goblet cells in the intestine [36]. Caudal-type homeobox protein 2 (CDX-2) is known to activate the *MUC2* promoter and is also upregulated during BE development [37, 38]. Therefore, CDX-2 upregulation may be interconnected with goblet cell differentiation via MUC2 expression during BE de-

velopment. Indeed, CDX-2 overexpression in vitro increases MUC2 mRNA and protein expression [39, 40]. Besides, positive MUC2 expression in non-goblet squamous epithelial cells has been shown to be valuable for detecting goblet cells in BE tissue sections of endoscopic biopsies [34]. Thus, the assessment of MUC2 may be useful for diagnostic purposes since the distribution of goblet cells is patchy in some cases.

Moreover, BACs segregate into gastric-type tumors with MUC5A positivity and intestinal-type tumors with MUC2 positivity; Szachnowicz et al. [41] suggested that these 2 BAC subtypes may reflect 2 different potential cells of origin being involved in BAC carcinogenesis.

CDX-2 as a Potential Key Regulator during BE Development

The key regulators involved in BE development have not yet been determined in detail. One suggested key regulator is CDX-2, which is known to be involved in intestinal differentiation and proliferation [42, 43]. Indeed, BE lesions show increased CDX-2 levels compared to normal esophageal squamous epithelia [38, 44, 45], but CDX-2 levels decrease again with progression to BAC [44, 45].

Table 1. Putative key regulators involved in the development of BE

Regulator	Finding
FOXA2	increased expression in BE and overexpression in esophageal epithelial cells induced MUC2 [56]
CDX-2	upregulated in BE tissues [38, 44, 45], complex formation with SMAD4 [17] and induction of intestinal markers [39]
GATA6	increased expression in BE [57], and overexpression, together with <i>FGFR2IIb</i> , increased the anchorage-independent growth [58]
<i>HOXB</i> genes	higher levels in BE samples and overexpression increased columnar markers [61]
TLR4	overexpressed in BE, and activation led to NF- κ B signaling [62]

In addition, 3 single-nucleotide polymorphisms of the *CDX2* gene (rs4769585 and rs3812863) have been associated with a higher risk for BE [46, 47].

Identification of CDX-2 target genes by a study on overexpression in intestinal rat cells linked CDX-2 to the Notch pathway [48]; this has been also observed in human BE tissue specimens [32]. ChIP-Seq analyses in the colorectal cancer cell line Caco-2 linked CDX-2 binding to target genes or their enhancers, involved in differentiation and transcriptional regulation [49]. Interestingly, enhancers are thought to define cell-specific transcription patterns [50]. Hence, the notion that CDX-2 overexpression leads to reshaping of enhancer activity appears feasible.

However, overexpression of CDX-2 in vitro [51] and in vivo [52] failed to induce complete intestinalization of normal esophageal squamous epithelial cells. Overexpression of CDX-2 in non-neoplastic esophageal epithelial Het-1A cells was found more successful in the induction of intestinalization [39] when compared to a similar study on EPC-hTERT cells [51]. Het-1A cells are immortalized by SV40 T-antigen [53], whereas EPC-hTERT cells are immortalized by hTERT [54]. hTERT does not disrupt cellular signaling compared to the immortalization by the SV40 T-antigen, which impedes the p53 and Rb pathways [55]. This may illustrate that BE development is implemented in complex cellular signaling networks and that overexpression of a single transcription factor may not be sufficient for intestinalization. Indeed, Mari et al. [17] recently showed that the complex formation of CDX-2 with SMAD4 is essential for the induction of intestinalization.

New and additionally regulators have therefore to be considered. For example, BE tissue samples show higher FOXA2 expression than normal esophageal squamous epithelia and, indeed, FOXA2 overexpression in esophageal squamous NES-B3T cells results in MUC2 expression [56]. Similarly, GATA6 expression is also increased in BE lesions compared to normal esophageal epithelium [57] and the overexpression of GATA6, together with FGFR2IIb, has been found to increase the anchorage-independent growth of the BE cell line CP-A [58]. Conflicting results show that upregulated miR-145 in BE lesions downregulates GATA6 and inhibits proliferation in non-neoplastic esophageal Het-1A cells [59]. Interestingly, it is thought that the binding of FOXA and GATA factors, which are pioneer transcription factors, opens up and reshapes closed (hetero)chromatin [60]. Hence, (over)expression of FOXA2 or GATA6 might induce embryonic development programs, leading to the rebuilding of the normal esophageal tissue architecture.

Further, *HOXB* genes are also overexpressed in BE and have been shown to influence the expression of columnar cell markers like MUC2 or keratin-18 without influencing CDX-2 levels [61]. Expression of Toll-like receptor 4 (TLR4) also increases during BE development and its activation via lipopolysaccharide increases NF- κ B activation and COX-2 expression [62]. Hence, several potential key regulators have been discovered in (over)expression studies on BE tissues/cells (table 1), but the functional consequences are mostly unknown and need further characterization.

Microenvironmental Changes during BE Development

For the development of BE lesions, chronic tissue damage from gastroesophageal reflux disease (GERD) seems to be critical and is the highest risk factor for BE [63]. Indeed, GERD leads to an aggressive environment with acidic conditions and reactive oxygen species [64], which may cause chronic inflammation. BE biopsies show an accumulation of oxidative DNA damage with subsequent induction of telomerase activity and *TP53* mutations [65]. It has thus been discussed whether BE development is a protective mechanism in the normal esophageal epithelium that establishes resistance to an acidic environment, e.g. by the induction of claudin-18 expression [30].

In vitro, acidic and/or bile acid treatment of non-neoplastic Het-1A cells results in increased CDX-2 expres-

sion via promoter demethylation [39]. Moreover, acidic treatment of Het-1A cells has been found to activate Hedgehog signaling with subsequent bone morphogenetic protein 4 (BMP4) expression and intestinalization [66]. Hence, microenvironmental stresses have been linked to epigenetic changes and the upregulation of the known BE key regulator, CDX-2.

BE is often associated with inflammatory processes, as reviewed by Poehlmann et al. [67]. The proinflammatory genotype based on the IL-12B C-allele with an associated higher expression of IL-12p70 predisposes for BE development [68]. Indeed, BE patients show an increased histopathological degree of inflammation from distal to proximal, accompanied by increased expression of cytokines like interleukin (IL)-1 β , IL-10 and IL-4 [69]. Interestingly, IL-1 β expression is markedly enhanced upon coculture of normal squamous epithelial and BE cells [69], suggesting an interaction of these two cell types. IL-10 expression is restricted to the inflammatory cells in nondysplastic BE lesions, but can also be observed in the BE cells themselves in dysplastic BE lesions [69]. Importantly, IL-10 as anti-inflammatory cytokine is thought to be involved in cancer immune evasion [70], and so increased IL-10 expression might contribute to neoplastic progression to BAC. In vitro IL-4 treatment of normal human esophageal epithelial cells increases expression of columnar markers like keratin-8, with the concomitant downregulation of squamous markers like keratin-13 or involucrin [71], indicating a potential trans-differentiation into a BE-like state. Moreover, BE lesions show higher levels of IL-6 [72] and, indeed, transformed BE cells start the secretion of IL-6 in vitro with the autocrine activation of the STAT3 axis, mediating resistance to apoptosis [73].

Several chemokines of the chr4q21 chemokine cluster are upregulated in dysplastic BE lesions via promoter demethylation [74], suggesting that epigenetic changes are involved in the cytokine changes observed during BE development.

A stromal microenvironment may also contribute to BE development. Saadi et al. [75] showed that expression profiles of the stromal components (after laser microdissection) are sufficient to discriminate between BE without dysplasia, BE with dysplasia and BAC. Moreover, increased expression of stromal genes, including *TMEPA1* and *JMY*, are significantly associated with a worse prognosis [75]. Stromal cells in BE tissue specimens also show increased BMP4 expression [66, 76]. Interestingly BMP4 stimulation is essential to induce MUC2 expression in CDX-2-overexpressing cells in vitro [17].

Thus, alterations of cytokine expression either in the stromal components or in the BE cells themselves might contribute to the cellular changes and even to neoplastic progression.

Epigenetic Alterations of BE Lesions

Since epigenetic alterations are described for BE lesions, it seems likely that epigenetic alterations are involved in BE development. Epigenetic mechanisms include noncoding RNA metabolism, DNA methylation and histone modifications. Indeed, many epigenetic aberrations have been described for BE lesions as well as for the progression from BE to BAC [77–79].

Changes in miRs have been extensively studied for BE lesions and its progression to BAC [77, 78]. This revealed, for instance, the progressive upregulation of miR-21 [80] or miR-221/222 [81]. Indeed, miR-221/222 has been shown to interact with the 3' UTR of the p27Kip1 mRNA, causing the proteasomal degradation of CDX-2 [81]. This partially explains the progressive downregulation of CDX-2 during BAC progression. Moreover, inhibitors of miR-221/222 impair tumor growth in xenografts [81]. Matsuzaki et al. [81] demonstrated that miR-221 and miR-222 are upregulated by bile acids via activation of the bile acid receptor.

DNA methylation [79] has also been a focus of BE research. For example, DNA hypermethylation frequently downregulates *CDKN2A* early in BE development and is also associated with a higher risk for progression to BAC [82–84]. Other markers for risk stratification of a progression to BAC are the silencing of *TIMP3* or *TERT* by DNA hypermethylation [85]. However, in general, DNA hypomethylation seems to be more common in BE lesions and leads to the expression of genes associated with differentiation programs, like the *HOX* genes, or cellular proliferation [86]. Interestingly, in vitro and in vivo studies show that DNA demethylation by 5-azacytidine or decitabine enhances intestinalization effects after CDX-2 overexpression [51, 52]. This may support the idea that DNA methylation changes are involved in BE development. However, it is still unclear what causes such changes in vivo. Besides, the assessment of DNA methylation in a subset of 8 genes (*p16*, *RUNX3*, *HPP1*, *NELL1*, *TAC1*, *SST*, *AKAP12* and *CDH13*) may be useful for the risk stratification of neoplastic progression [87]. As an adjunct to histopathology, this may be helpful in improving patient selection for surveillance endoscopy, since annual conversion rates of BE are relatively low [8, 9], and it has

Table 2. Overview of established BE cell lines

	Parental cell line	Source	p53 status	p16 status	Chromosome
CP-A	KR-42421 [89]	nondysplastic BE [88]	WT (exon 5–8) [88]	del(C5.1) [88]	50 [88]
CP-B	CP-52731 [89]	H-IEN [88]	A175H [88]	L97G [88]	44 [88]
CP-C	CP-94251 [89]	H-IEN [88]	A248T [88]	WT [88]	44 [88]
CP-D	CP-18821 [89]	H-IEN [88]	exon 8/302 frameshift [88]	del(C5.1) [88]	41 [88]
BAR-T	–	nondysplastic BE [90]	functional intact, not sequenced [90]	functional intact, not sequenced [90]	46 [90]
BAR-T9	–	nondysplastic BE [91]	–	–	–
BAR-T10	–	nondysplastic BE [91]	–	–	–

H-IEN = High-grade intraepithelial neoplasia.

not yet been defined which patients benefit from regular surveillance [13].

Histone modifications have not been studied in cases of BE or BAC, but predominantly in ESCC cases [77]. However, *HOXB* genes show a loss of the repressive H3K27me3 marker and an increase in 2 activating histone marks (H3K4me3 and global histone H3 acetylation) in BAC when compared to normal esophageal squamous epithelium [61]. Clearly, histone modifications need to be further investigated to clarify their role in BE development.

Experimental Systems for BE Research

Cell line systems would theoretically provide ‘unlimited material’ for in vitro studies. Currently, there are only 2 immortalized cell line sets available: the CP-A/CP-B/CP-C and CP-D cell lines and the BAR-T cell lines, which have all been immortalized by hTERT [88–91]. Table 2 summarizes the characteristics of these established BE cell lines. These BE cell lines provide a useful tool for BE research. Analysis of acid treatment in BAR-T cells revealed an increase in reactive oxygen species, DNA damage and Chk2 activation, proposing acid to be a potential carcinogen [64, 91]. Importantly, BAR-T cells have also been used to engineer sub-cell lines with the knockdown of p53 and/or introducing oncogenic H-Ras^{G12V} to recapitulate the different stages of BE progression to BAC [92]. Bus et al. [93] recently summarized the findings on the cellular effects of bile and/or acid treatments in BE cell lines as well as in non-neoplastic esophageal cell lines. Primary cell cultures of BE-derived cells could be cultivated for at least 17–29 population-doublings before reaching senescence [88], but these primary cell lines were then mainly used to generate the immortalized CP cell lines [89].

Classic cell culture approaches with monolayer systems may not be ideal to study the complex 3-dimensional changes in tissue architecture during BE development. Indeed, organoid cultures of primary BE patient material were successful [94], providing a 3-dimensional in vitro model. This was also applied to mouse esophageal epithelial cells, showing comparable differentiation of such organoids in vitro and the esophageal squamous epithelium in situ based on the expression patterns of cytokeratin-17 and p63 [95]. By using organoids with mouse keratinocytes in vitro, DeWard et al. [95] showed that Sox2, Wnt and BMP signaling regulated the self-renewal of putative esophageal stem cells. However, organoids have not been applied to model BE development.

Monolayer cell cultures as well as organoid cultures are missing stromal interactions, which have been reported to influence BE development as mentioned above. Hence, organotypic cultures (OTCs) using esophageal fibroblasts in the matrix layer may be more suitable to mimic interaction with the microenvironment. OTCs have been established for non-neoplastic esophageal epithelial cells and BE cell lines [96]. However, Kosoff et al. [96] reported invasive growth of the CP-A cells in OTC. Thus, established BE cell lines may not mirror the noninvasive parental BE lesions (maybe through immortalization) and this has to be carefully considered when designing studies and interpreting data. OTCs of non-neoplastic Het-1A cells have been used to gain first insights into trans-differentiation processes, showing that *c-myc* and *CDX1* coexpression induce MUC5A expression [97]. Hence, in view of the complex cellular involvement, difficulties still exist in modelling the 4 routes to BE development, and we await the further exploration of this system.

BE mouse models are rare and are mostly based on surgical approaches. Such approaches are very laborious

and can be ineffective. For example, in some models, only 7% of the mice developed BE lesions with no progression to BAC [98]. However, other surgical mouse models showed higher rates of BE lesions and progression to BAC in 55% of mice after 5 months [99].

A genetic mouse model for BE/BAC was developed by the overexpression of human IL-1 β , resulting in the L2-IL-1 β mouse [25]. These mice developed Barrett-like metaplasia in the esophagus, resembling human BE lesions, and around 20% developed high-grade dysplasia or even intramucosal BAC at around 20 months of age. A transgenic mouse model of CDX-2 (over)expression in the esophageal epithelium under control of the keratin-14 promoter failed to induce complete intestinalization and showed only minor morphologic changes for the basal cell compartment [52].

Importantly, surgical and genetic mouse models have the drawback of histological differences to the situation in humans, e.g. the lack of submucosal glands [100]. This may exclude some potential human BE development routes a priori, like the trans-commitment of stem cells derived from esophageal glands.

For gaining insights in some side aspects of BE development, an alternative could be virtual/mathematical computer models which lack, of course, the complexity of biological networks/microenvironments. However, they do allow the modelling of the evolutionary processes during cancer initiation, like cellular competition due to mutations, leading to a higher mutational rate or growth advantages (e.g. the cumulative probability of mitosis in single cells) [101]. Indeed, virtual esophageal cancer models have been the first to show that the generation of a genetically unstable cell clone increases the probability of progression to esophageal cancer, which has been partially demonstrated in a BE patient cohort [101, 102].

Conclusion

The Barrett mucosa is a striking and complex pathological lesion, arising from a switch in cellular differentiation. The exact molecular pathomechanisms leading to a Barrett mucosa are only poorly understood. But 4 potential routes to the formation of BE lesions have been suggested, including trans-differentiation, trans-commitment of esophageal stem cells, colonization by bone marrow-derived cells and cellular competition. Clearly, future work is needed to clarify these hypotheses, taking into consideration the individual patient's background

and to establish whether the pathomechanisms are mutually exclusive.

Moreover, the key drivers of BE development are largely unknown. So far, experimental overexpression of the potential morphology/differentiation-associated regulatory protein CDX-2 shows only limited effects, and thus further key regulators for the induction of intestinalization have to be identified. For example, the pioneer transcription factors, such as FOXA2 and GATA6, have been recently reported to be overexpressed in BE lesions. Investigation of their functional consequences appears to be the next experimental step forward.

Clearly, the microenvironment is also involved in BE development. This has already been shown for stromal components and for the metaplastic cells themselves. Altered expression of cytokines might contribute to trans-differentiation (e.g. IL-4) or even neoplastic progression (e.g. IL-10).

Future concepts and experimental strategies should include the investigation of epigenetic alterations, especially histone modifications, which have not been studied so far. Therefore, translational approaches, based on (1) prospective biomarker studies which account for morphological and molecular characteristics of tissue specimens, (2) in vitro experiments and (3) new robust animal models, will facilitate novel insights into the causes and consequences of BE.

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