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Today's Mistakes and Tomorrow's Wisdom in Development and Use of Biomarkers for Barrett's Esophagus

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Keywords

Barrett's esophagus · Biomarker · Risk prediction · Esophageal adenocarcinoma · Genomics

Abstract

Background: A histological diagnosis of dysplasia is our current best predictor of progression in Barrett's esophagus (BE), the precursor of esophageal adenocarcinoma (EAC). Despite periodic endoscopic surveillance and assessment of dysplastic changes, we fail to identify the majority of those who progress before the development of EAC, whereas the majority of patients undergo endoscopy without showing progression. ***Summary:*** Low-grade dysplasia (LGD), confirmed by expert pathologists, identifies BE patients at higher risk for progression, but the diagnosis of LGD is challenging. Recent research indicates that progression from BE to EAC is heterogeneous and can accelerate via genome doubling and genome catastrophes, resulting in different ways to progression. We identified 3 target areas, which may help to overcome the current lack of an accurate biomarker: (1) the implementation of somatic point mutations, chromosomal alterations, and epigenetic changes (genomics and epigenomics), (2) evaluate and develop biomarkers over space and time, (3) use new sampling methods such as non-invasive self-expandable sponges and endoscopic brushes. This review focus on the state of the art in risk stratifying BE and on recent advances which may overcome the limitations of current strategies. ***Key Messages:*** A panel of clinical factors, genomics, epigenomics, and/or proteomics will most likely lead to an assay that accurately risk stratifies BE patients into low- or high-risk for progression. This biomarker panel needs to be developed and validated in large cohorts containing a sufficient number of progressors, with testing samples over space (spatial distribution) and time (temporal distribution). For implementation in clinical practice, the technique should be affordable and applicable to formalin-fixed paraffin-embedded samples, which represent standard of care.

Introduction

Barrett's esophagus (BE) is the precursor of esophageal adenocarcinoma (EAC), which has a poor 5-year survival rate of approximately 20% [1]. Because early cancer is amenable to endoscopic treatment, periodic surveillance endoscopy is recommended in BE.

A perfect biomarker accurately identifies those at high risk before progression to cancer to allow for an intensified surveillance or even preventive treatment. On the other hand, a biomarker ideally also identifies individuals at low risk for progression, sparing them from intensified endoscopic surveillance programs and consecutively leading to decreased morbidity and costs.

Similar to the colon, progression from BE to EAC was considered to occur in a stepwise cascade from specialized intestinal metaplasia without dysplasia, followed by low-grade dysplasia (LGD), and subsequently, high-grade dysplasia (HGD) and invasive cancer. This ideally results in a long "window of opportunity" to identify and

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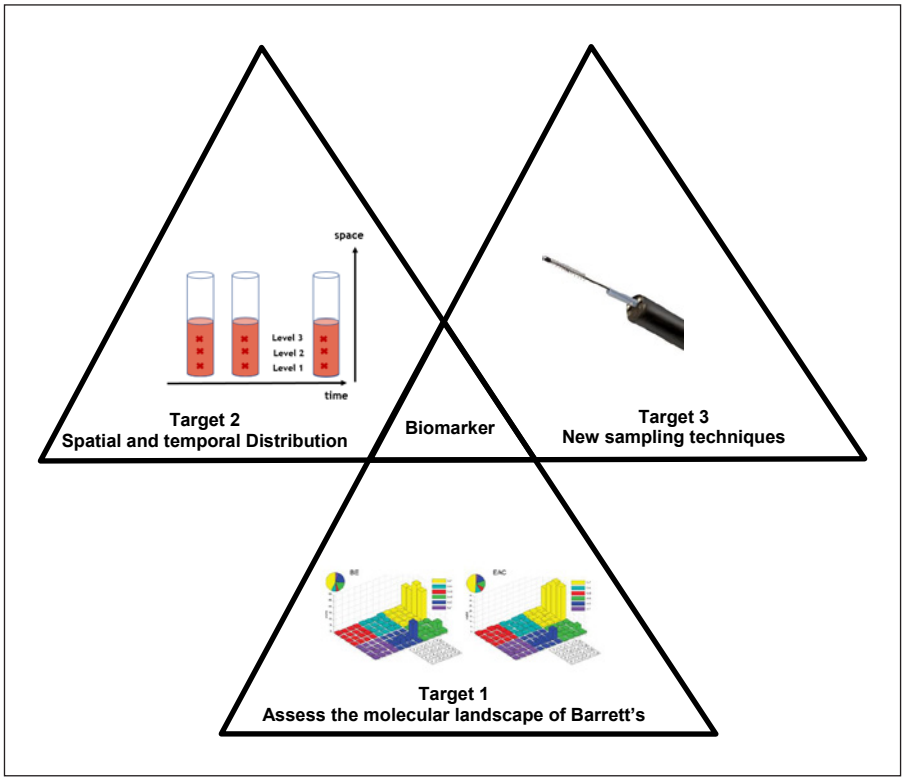

Fig. 1. Illustration of three target areas to overcome the current lack of an accurate biomarker predicting progression in Barrett's patients. Target 1, the ideal set of molecular biomarkers needs to be determined. Target 2, how these biomarkers perform in spatially and temporally distinct samples will be needed to inform the timing and number of samples needed for clinical testing. Target 3, new sampling devices that sample larger areas of tissue and/or are less invasive may improve biomarker testing.

treat dysplastic precursor lesions before the development of cancer [2]. Despite established surveillance programs for BE, we still fail to identify the majority of EAC early, while they are amenable to endoscopic treatment. This had led others to speculate that either progression occurs faster than originally thought or alternative progression pathways exist.

To be successful, biomarkers should be superior to the current diagnostic and prognostic approach and be practical and cost-effective for implementation in clinical practice. We identified three target areas, which may help to overcome the current lack of an accurate biomarker predicting progression in BE patients (Fig. 1). First, recent technological and computational advances allow the identification of somatic point mutations, chromosomal alterations, and changes in protein-expression in BE and EAC clinical samples. Second, testing samples over space (spatial) and time (temporal) will improve the understanding of cancer evolving in a 3-dimensional way (as it is in the esophagus) and will identify both changes which occur early and late in the cascade from BE to EAC. Third, new sampling methods such as noninvasive methods using self-expandable sponges/balloons and endoscopic brushes may overcome the limitations of current 4-quadrant sampling according to the Seattle protocol. This review focus on the state of the art in risk stratifying BE and on recent advances which may overcome the limitations of current strategies.

State of the Art and Todays Mistakes

Demographic Factors and BE Associated Factors

Demographic and endoscopic factors are attractive risk predictors, since they are easily available and applicable to the majority of patients. Increasing age has been showed to be associated with an increased risk of progression in a recent meta-analysis; however, this association did not remain statistically significant in a more restricted analysis including only studies that reported multivariate analysis adjusted for age, sex, dysplasia, and BE length [3]. Additionally, the value of age is a questionable risk factor, since the low progression rate of 0.12–0.78% per patient-year needs to be weighted carefully against the invasiveness and costs of endoscopic surveillance in elderly and frail BE patients [4, 5].

Male sex is associated with an increased risk of progression, including in multivariate analysis adjusted for other clinical and demographic factors. This is in line with a strong male predominance in patients with EAC. A recent sex-specific analysis by Dong et al. [6] identified various independent genetic loci in males only including variants in 12p12.3. This variant encodes for an enzyme (microsomial gluthatione S-transferase 1, MGST1) linked to tumorigenesis and apoptosis and has been shown to be associated with an increased risk of BE and EAC in European populations [7]. In a Mendelian randomized analysis using data from patients with EAC and controls, high-

er genetically predicted levels of follicle-stimulating and luteinizing hormones were associated with higher and lower risks of EAC in men, respectively [8]. Additionally, several studies in mouse models indicate that a combination of clinical risk factors such as gastro-esophageal reflux, bile acids, the microbiome, and a high-fat diet promotes inflammation that disrupts stem cell homeostasis leading to malignant progression [9, 10].

LGD and p53 Immunohistochemistry

Whereas patients with nondysplastic (ND) BE carry a very low risk for progression (0.12–0.78%/year), the progression rate increases substantially in the presence of a histologic diagnosis of LGD [4, 5]. However, the diagnosis of LGD is challenging due to several reasons. This includes varying histological criteria defining LGD and a lack for a threshold to distinguish reactive atypia from true dysplastic changes. Thus, the diagnosis of LGD is subjective and results in a significant inter- and intra-observer variation in diagnosing LGD among pathologists as well as widely varying progression risks [11]. Consequently, the majority of community-based LGD diagnosis is downstaged to ND if reviewed by an expert pathologist and only expert confirmed LGD harbors a markedly increased annual progression risk, found to be 9.1% per patient-year in one study [12]. Additionally, two Dutch studies demonstrated an increasing progression rate if more than one pathologist confirmed the diagnosis of LGD and if LGD was confirmed in a subsequent endoscopy [12, 13].

According to the British Society of Gastroenterology, the addition of p53 immunohistochemistry (IHC) to the histopathological assessment may improve the diagnostic reproducibility of a diagnosis of dysplasia and should be considered as an adjunct to routine clinical diagnosis [14]. Aberrant p53 staining has repeatedly shown to be associated with progression to dysplasia and EAC, as recently published in a meta-analysis by Snyder et al. [15]. However, the identification of p53 abnormalities may be identifying risk of progression independent of dysplasia status as several more recent studies have identified p53 alterations in ND samples from patients who eventually progress [16, 17]. Regardless, the application of p53 IHC is limited by similar conditions as the histopathological assessment of dysplasia. The interpretation of the intensity of p53 IHC staining is subjective, leading to an interobserver variability between pathologists. To be implemented in future guidelines, similar to HER2 IHC, a standardized and/or automatized interpretation of the staining will be mandatory.

Despite intentions to "purify" the population of patients diagnosed with LGD by establishing pathology training programs to harmonize the heterogeneous diagnosis of LGD, the limitations of LGD as a biomarker remain a problem [18]. Recent research indicates that progression from BE to EAC is heterogeneous and can accelerate via genome doubling and genome catastrophes, resulting in three, not mutually exclusive, main roads to progression [17, 19, 20]. The initial progression (country road) is a stepwise and slow progression via dysplasia to EAC by accumulating different types of driver mutations favored by environmental risk factors. On the other hand, catastrophic events such as chromothripsis (expressway) and whole genome doubling (fastlane) can occur at any stage and lead to dramatically accelerated progression. This results in a shorter "window of opportunity" for early detection and requires shorter endoscopic surveillance intervals. Recent technological advances allow the detailed assessment of the genomic landscape of BE and will continuously improve our understanding of the origin and progression in BE. This is underlined by a very recent publication indicating that undifferentiated gastric cells from the cardia precede Barrett's and EAC, regardless whether metaplasia precursors were identifiable histologically, and similar results were shown earlier in studies using preclinical mouse models [9, 21]. A different study evaluated a specific mouse model of BE resulting in inflammation with development of metaplasia and progression to dysplasia. Using these mice, abberant activation of Notch signaling and its influence on the malignant cascade were studied. Expression of NOTCH2 in Lgr5+ cells resulted in reduced goblet-like cell maturation and accelerated development of cancers in the squamocolumnar junction, whereas mice with deletion of NOTCH from Lgr5+ cells had increased maturation of goblet-like cells and developed fewer cancers. Interestingly, this inverse correlation of upregulation of Notch signaling with decreased goblet cell differentiation and accelerated development of cancers could be observed in humans using a prospective, multi-center cross-sectional study of 164 patients with BE (with and without dysplasia and cancer) and non-BE controls [22].

Omics to Understand the Genomic Landscape of Progression

Genomics

Genomic alterations include the evaluation of focal mutations and larger structural alterations such as aneuploidy and specific chromosomal alterations including copy-number changes (deletions and amplifications).

Focal Somatic Mutations

The identification of somatic alterations required for progression from BE to EAC (i.e., inactivation of tumor suppressor genes and activation of oncogenes) are logical biomarkers and have the potential to assist in identifying

precancerous lesions at the highest risk for progression. Stachler et al. [17] identified a *TP53* mutation in 11 of 24 BE patients (46%) prior to progression to cancer, but only in 4 (5%) of the patients without progression. In a majority (80%) of samples with a *TP53* mutation the expert histologic diagnosis was nondysplastic BE (NDBE), indicating that this alteration occurs early in the progression cascade. Additionally, total mutational burden and mutations in *ARID1B*, *APC* ,and *ERBB2* were significantly more frequent in progressors compared to nonprogressors; however, at a lower frequency ranging from 12.5 to 33.3%. Mutations in *PIK3CA* and *CTNNB1* have been found in BE in an earlier publication by the same group [23]. The results of frequent *TP53* mutations in ND samples up to 9 years prior to progression are remarkable, since older studies failed to identify recurrent *TP53* mutations in NDBE progressing to cancer [24]. Less sensitive genomic assays and the selection of patients without future progression are possible explanations for the contradictory results. Two germline variants in CDKN2A (rs2518720 and rs3088440) were found to be independently significantly associated with reduced risks of progression in a prospective cohort of 408 patients with BE [25]. Expression of the two variants reduced microRNA-mediated repression of the CDKN2A mRNA, a well-known tumor suppressor gene. Besides *TP53*, given the lower overall rate of any one specific somatic mutation, using mutations for biomarker-based risk stratification will likely require a panel of multiple genes.

Mutational Burden and Clonal Diversity

Overall, a low frequency of recurrent somatic mutations is observed, and BE harbors a number of point mutations even in cases that never progress to cancer. Therefore, recent studies focused on the total number of mutations (mutational burden). Again, Stachler et al. [17] identified 147 pathogenic mutations across 57 individual genes with a significant higher burden of total pathogenic mutations in progressors compared with patients showing no progression during long-term follow-up (2.5 vs. 1.2, $p < 0.001$). Clonal diversity assessed by the Shannon index was shown to be predictive of progression to EAC in several studies. Number of clones and genetic divergence based on *Loss of heterozygosity)* in p16 and p53 were strong predictors of neoplastic risk (RR 95% CI 1.43 [1.16–1.72] and 3.59 [1.36–9.45], respectively) [26].

Chromosomal Aberrations – Aneuploidy, Copy-Number Alterations, and Translocations

Aneuploidy is defined as an abnormal number of chromosomes in a cell and has been described very early as a potential biomarker for risk stratification in BE [27]. A recent study evaluated a panel of nine biomarkers including aneuploidy in a total of 127 BE patients who were followed, prospectively [28]. In 42 patients, progression to LGD (28%) or HGD and/or EAC (72%) was observed during endoscopic follow-up. Aneuploidy was the only predictor of histologic progression, having a 6.6-fold increased risk of progression compared with patients showing absence of aneuploidy. However, the sensitivity of the test was low (32%, 95% CI: 16–52), indicating that 1) a negative test does not allow to prolong surveillance intervals and 2) a combination with other biomarkers is warranted. One of the main issues in this study was that flow cytometry on fresh frozen biopsies was used to assess aneuploidy. This does not reflect the standard of care, as usually formalin-fixed paraffin-embedded biopsies (FFPE) are collected. However, application in FFPE biopsies is a key limitation for translation into clinical practice.

A recent study from Killcoyne et al. [19] studied genome-wide copy-number instability as a marker for risk of progression using shallow whole genome sequencing in a retrospective, case-control study. This approach has been optimized for the use in FFPE samples. Patients were classified as low, moderate or high risk for progression based on a combination of copy-number information and a measure of overall complexity. In progressing BE patients, a generalized disorder across the genomes was observed, and interestingly, this was independent from a diagnosis of dysplasia. In contrast, in patients without progression, few copy number alterations were observed, which allowed stratification between patients with and without progression. Whereas 60.5% of the samples without dysplasia that belonged to progressors were classified as high risk, 64.7% of the samples from nonprogressors were classified as low risk, resulting in a good predictive accuracy of the model (AUC 0.89) [19].

Several groups have used fluorescence in situ hybridization (FISH) to look at chromosomal copy number changes. Fritcher et al. [29] utilized a series of four FISH probes covering *MYC*, *CDKN2A*, *ERBB2*, and 20q13. They found the FISH probes had a sensitivity of 50% for LGD, 82% for HGD, and 100% for EAC in their limited cohort [29]. Timmer et al. [30] found a FISH panel of CDKN2A, TP53, ERBB2, MYC, 20q, centromeric chr 7, and centromeric chr 17 were able to predict progression with an AUC of 0.76 when combined with clinical risk factors of BE length and patient age in a prospective cohort of 428 BE patients (35 progressors to HGD or EAC).

Bajpai et al. [31] used FISH to evaluate chromosomal translocations in human EAC tissues. The identification of chromosome translocations or gene fusions is an interesting approach to identify a valuable biomarker. Although they may be less common in solid tumors, they are likely more specific and may serve, additionally, as potential therapeutic targets in the future. The authors refined a recurring chromosomal translocation (t(10:16),

which they described earlier in BE cells before they undergo malignant transformation as a three-way translocation between the chromosomes 2, 10 and 16) in FFPE samples from human EAC. The translocations occurred in the EAC samples, but not in normal esophagus or NDBE. While interesting, significant further research by testing multiple time points prior to progression in a larger sample set is needed to evaluate if and at which time point these translocations exactly occur, so they can serve as a reliable biomarker for risk stratification in BE carcinogenesis.

Epigenomics

Epigenetic changes summarize post-translational modifications such as hypo- and hypermethylation and acetylation which can result in gene silencing. The role of CpG island hypermethylation in tumorigenesis was described by Eads et al. [32], and underlines the potential of epigenetic changes since it occurs early in the malignant cascade. One challenge in methylation analysis is that it often requires more DNA than typical genomic tests. However, technological advances have improved the ability to perform these studies in smaller FFPE samples to translate epigenomics from the laboratory to the clinical practice. One of the first tumor suppressor genes described to be aberrantly methylated in BE was *CDKN2A*. *CDKN2A* promotor hypermethylation combined with 9p21 chromosomal loss leads to inactivation of this gene and has been described as an early event in BE pathogenesis [33]. However, there is ongoing debate as to how well *CDKN2A* will work as a biomarker as genomic and epigenomic *CDKN2A* alterations have also been frequently found in samples from nonprogressors. Jin et al. [34] reported an adjusted AUC of 0.732 for a panel of 8 methylation markers (*CDKN2A*, *RUNX3*, *HPP1*, *NELL1*, *TAC1*, *SST*, *AKAP12*, and *CDH1*) in a cohort of BE patients that included 50 progressors and 145 nonprogressors. Alvi et al. [35] reported a four-gene methylation panel including *SLC22A18*, *PIGR*, *GJA12,* and *RIN2*. The panel risk stratified BE patients into low, intermediate and high-risk for prevalent disease in a prospective cohort containing different histologic stages of BE and EAC.

Recently Moinova et al. [36] published a panel of methylated genes from samples collected with a swallowable balloon-based device identifying 96% of BE patients with dysplasia and with a high specificity. The combination of a panel of methylated genes and a noninvasive sampling technique is promising, but needs further validation in larger prospective cohorts.

Tissue Slide Based Approaches

As discussed above, there is growing evidence that p53 IHC as a surrogate for genomic alterations in *TP53* may serve as a biomarker for risk stratification. In a prospective cohort of BE patients, including patients with both NDBE and LGD, Kastelein et al. [16] found a significant elevated relative risk in patients with NDBE with abnormal p53 IHC and LGD with abnormal p53 IHC. Redston et al. [37] recently confirmed these results in a series of large retrospective and prospective cohorts. Others have tried to include other protein markers, but few have been validated across large cohorts [38]. Several studies have utilized a panel of 10 immunofluorescent markers p16, alpha-methylacyl-CoA racemase, p53, HER2, cytokeratin-20, CD68, cyclooxygenase-2, hypoxia-inducible factor 1-alpha subunit, and CD45RO, and Hoechst (nuclear labeling) and digital image analysis to risk stratify patients with BE. They have developed a risk classifier that provides a risk score with three categories (low-risk, intermediate-risk, and high-risk). In two of the more recent studies, they tested their classifier in a single-blinded, case-control study of BE and a retrospective cohort study of patients with a community diagnosis of LGD [39, 40]. In the case-control study, which included 58 progressors and 210 nonprogressors, they found a sensitivity of 29% and a specificity of 86% with a HR of 4.73 (CI 2.5–8.8). In the LGD cohort study (34 progressors and 121 nonprogressors), they found a sensitivity of 67.7% and specificity of 78.6% (Table 1).

Biomarkers Can Be Affected by Their Spatial and Temporal Distribution

An important potential reason why translation of biomarkers into clinical practice has been unsuccessful is the variability of biomarker expression across the surface of a BE segment (spatial distribution). If expression of a biomarker is highly variable across a BE segment, external validation of such a biomarker may fail if it is applied on single biopsies or single level biopsies from longer BE segment. Additionally, more insights in expression of biomarkers over time (temporal distribution) may be useful in personalizing surveillance intervals. If a biomarker predicts progression early and reliable with little variation over time, patients with a low risk of progression may undergo more lenient surveillance [41]. One of the first studies evaluating biomarker over space and time was published by Li et al. in 2014 [42]. Using fresh frozen samples from a longitudinal case-control cohort, they characterized somatic chromosomal alterations in 79 progressors and 169 nonprogressors. Whereas the genomes of nonprogressors largely remained stable over long periods, progressors developed chromosomal instability with initial gains and losses, genomic diversity, and selection of somatic chromosomal alterations resulting in catastrophic genome

Table 1. A selection of biomarker evaluating risk predictors (models) in BE including main results and limitations

Biomarker biology	Biomarker	Reference	Study design	Samples (case:control)	Results	Limitation(s)
Histology						
Dysplasia	Expert confirmed LGD	Duits et al. [12]	Retrospective	255 (45:210)	*1 expert* OR 3.8 (0.9–16.0) *3 experts* OR 38.8 (10.7–140.5)	Retrospective Interobserver variability
Protein-expression						
IHC	P53	Kastelein et al. [16]	Retrospective	720 (49:586)	*Overexpression* RR 5.5 (3.1–10.0) *Loss of p53* RR 13.4 (5.1–35.3)	Retrospective Interobserver variability
	P53	Redston et al. [37]	Prospective	1,438	*ABNL TP53 in ND* HR 12.5 (8.0–19.6)	Heterogenenous cohorts
TissueCypher	Combination of 9 markers + morphology	Davison et al. [39]	Retrospective	268 (58:210)	*High-risk in ND* HR 5.1 (2.1–12.3)	Retrospective One time point
		Frei et al. [40]	Retrospective	155 (34:121)	*High-risk in LGD* HR 6.7 (3.2–13.8)	Retrospective One time point
Genomics						
Somatic mutations	TP53	Stachler et al. [17]	Retrospective	97 (24:73)	OR 13.8 (3.2–60.5)	Sample size One time point
	CDKN2A (SNP)	Buas et al. [7]	Prospective	413	HR 0.5 ($p = 0.009$)	One time point
Clonal diversity	Shannon diversity LOH index	Maley et al. [26]	Prospective	268	RR 11.0 (5.8–21.0)	Fresh-frozen biopsies
Aneuploidy	Aneuploidy	Hadjinicolaou et al. [28]	Prospective	127 (42:85)	OR 6.6 (1.8–24.8)	Fresh-frozen biopsies
	Panel of 5 chromosomal CNs	Douville et al. [45]	Cross-sectional	268 (111:157)	BAD-classifier AUC 0.868	Fresh-frozen samples
Copy-number alterations	Panel of 29 CN alterations	Li et al. [43]	Prospective	248 (79:169)	3-tier classification AUC 0.94	Fresh-frozen samples
	Genome-wide CN instability + measure of overall complexity	Killcoyne et al. [19]	Retrospective	75 (18:58)	3-tier classification AUC 0.89	Short follow-up
Epigenomics						
Methylation	Panel of 4 genes with methylation	Alvi et al. [35]	Prospective	98 (20:78)	3-tier classification AUC 0.988	One time point Heterogeneous cohorts

ABNL, abnormal; CN, copy-number; HR, hazard ratio; LOH, loss of heterozygosity; OR, odds ratio; RR, relative risk; SNP, single-nucleotide polymorphism.

doubling [43]. Recently, Killcoyne et al. [19] further developed this model by evaluating a prediction model using copy-number alterations in FFPE samples originating from multiple levels per endoscopy and multiple subsequent time points in both progressors and nonprogressors. For patients that progress, 50% (8/16) of endoscopies had at least one sample classified as high risk 8 or more years prior to EAC. Cases which lack early high risk patterns of progression acquired these over the following years, leading to 78% of endoscopies with at least one high risk sample 1 year prior to progression [19]. In a retrospective cohort study, Frei et al. [41] investigated ND samples in a nested case-control study using a Tissue Systems Pathology Assay. This risk prediction assay uses a multiplexed fluorescence imaging platform that automatically extracts quantitative data on multiple tissue biomarkers. The assay identified 31% of progressors when assessing a ND single biopsy level at least 2 years prior to progression. Interestingly, sensitivity increased to 50% when multiple levels were assessed. This underlines the importance for future research to either identify a biomarker represented in the majority of the BE segment or to collect material from a wider area than with four-quadrant forceps biopsies, which are prone to sampling error [41].

Wide Area Sampling May Overcome the Limitations of Forceps Biopsies

Traditionally, studies evaluating biomarkers in BE are using samples collected by forceps biopsies during endoscopic surveillance, as this is current standard clinical practice. However, taking 4 biopsies every 1–2 cm in the endoscopic visible BE, according to the Seattle protocol, is time consuming, and the adherence outside from study protocols or tertiary referral settings is low. Consequently, the probability to miss prevalent dysplasia, our currently best biomarker, is increased.

A very logical concept is to collect material using either a brush (requiring endoscopy) or nonendoscopic techniques using swallowable devices such as self-expandable sponges or inflatable baloons. These techniques incorporate the advantage of collecting material from the entire esophagus, in contrast to a punctual assessment by forceps biopsy.

The wide area transepithelial sampling device using 3-Dimensional computer assisted analysis (WATS-3D) is a brush that collects material from the deeper (transepithelial) layers and allows broader areas to be sampled. Several studies showed an increased diagnostic yield for identifying additional BE patients with dysplasia, however only if the WATS-3D was used as an adjunct to conventional 4-quadrant biopsies according to the Seattle protocol [44]. Although the concept of the device seems to be very logical, the results are not as promising as expected, mainly due to some methodological flaws in the study design. The possibility of combining WATS-3D and genomic biomarkers to improve the diagnostic and predictive accuracy for patient risk stratification is an area for evaluation in future studies.

Douville et al. [45] obtained samples from a cross-sectional cohort of BE patients with and without dysplasia and EAC using esophageal brushings. Combining esophageal brushing with a PCR-based massively parallel sequencing assay they identified both global and individual chromosome alterations. A 3-tier classifier (Barrett's Aneuploidy Decision [BAD]) based on 6 specific chromosomal alterations (1q gain, 9p loss, 12p gain, 17p loss, 20q gain, and focal amplifications of 8q24) was developed. Not-BAD cases indicated relative nonaneuploidy, maybe-BAD cases indicated a greater potential risk of progression and very-BAD cases had losses of 9p or 20q, gains of 1q, 12p or 20q or a focal gain of 8q24. The BAD classification of DNA from esophageal brushings as evaluated in the validation set was highly correlated with the histopathologic classification of the same patients, as 96.4% of the EAC cases were classified as very-BAD, and 63.4% of the ND patients as not-BAD. A limitation of this study is the cross-sectional design and the limited follow-up, which may partly explain the relatively high number of NDBE cases scoring either very- (7.3%) or maybe-BAD (29.3%).

In a multicenter cohort study (BEST2) published by Ross-Innes et al. [46], a nonendoscopic device (cytosponge) was coupled with clinical and molecular biomarkers. A panel consisting of three protein biomarkers (p53, c-Myc, and Aurora kinase A), two methylation markers (MYOD1 and RUNX3), glandular atypia and TP53 mutation status was developed in a discovery cohort and validated in a second cohort consisting of 65 patients. Based on a 3-tier classifier, 25 (38%) of the patients were classified as low risk, and the probability of absent dysplasia was 96.0% (99% CI 73.80–99.99). The high risk group (8%), consisted of no ND cases and 5 patients with HGD. Although this panel does not discriminate the patients at need for endoscopic treatment, it identifies patients at low risk for progression in whom endoscopy could be avoided.

One complicating factor for many of these devices that sample a broader surface area is that they also have the potential to sample the squamous esophagus. This excess of non-BE cells has the potential to dilute genomic or epigenomic biomarkers found within the BE cells to the point where they are no longer easily detected. Additionally, the normal squamous epithelium in the esophagus is known to harbor multiple genomic alterations which may complicate assay interpretation. The recently introduced balloon device from Lucid Diagnostics (NY, New York, USA) that can be deflated and retracted into the airline to protect the sampled BE cells as it passes the squamous upper esophagus and has the potential to mitigate these issues.

Future Directions

The genomes of BE and EAC share a high number mutations, but are heterogeneous with a low recurrence of somatic mutations in any given gene except *TP53*. Different pathways to progression seem to exist. Whereas in some patients disease progression is slow with cumulating point mutations in tumor suppressor genes, other tumors show punctuated evolution resulting in rapid progression. These pathways share an abnormal copy-number profile with amplifications, deletions, and complex rearrangements, which makes the assessment of copy-number alterations a valuable target for biomarker development. Due to the heterogeneous nature of BE and EAC, a panel of some combination of clinical factors, genomics, epigenomics, and proteomics will most likely lead to a biomarker assay which accurately risk stratifies BE patients into low- or high- risk for progression. Large studies comparing the different biomarkers are needed in order to identify the best combination toward clinical implementation. The biomarker panel will need to be developed and

validated in large cohorts containing a sufficient number of progressors, while testing samples over space (spatial distribution) and time (temporal distribution). To be implemented in clinical practice, the technique should be affordable and applicable to FFPE samples which represent standard of care until other sampling devices prove to be significantly superior. While molecular biomarkers seem to be a major way forward in risk prediction, fully understanding the conditions and source of BE formation and progression may also lead to new insights in prevention and risk stratification. There is growing evidence that stem like cells within the gastric cardia or gastroesophageal junction can proliferate and differentiate to form BE [9, 21]. How this occurs and what clinical/environmental factors (such as male sex, the microbiome, and GERD) can disrupt the normal cellular states and promote this process will be important areas of future studies. If this process is better understood, new therapies aimed at preventing progression may be possible.

Conflict of Interest Statement

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References

1 Zhang Y. Epidemiology of esophageal cancer. World J Gastroenterol. 2013;19:5598.
2 Reid BJ, Paulson TG, Li X. Genetic insights in Barrett's esophagus and esophageal adenocarcinoma. Gastroenterology. 2015;149:1142–52.e3.
3 Krishnamoorthi R, Singh S, Ragunathan K, Visrodia K, Wang KK, Katzka DA, et al. Factors associated with progression of Barrett's esophagus: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2018;16:1046–55.e8.
4 Hvid-Jensen F, Pedersen L, Drewes AM, Sørensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. N Engl J Med. 2011;365:1375–83.
5 Klaver E, Bureo Gonzalez A, Mostafavi N, Mallant-Hent R, Duits LC, Baak B, et al. Barrett's esophagus surveillance in a prospective Dutch multi-center community-based cohort of 985 patients demonstrates low risk of neoplastic progression. United European Gastroenterol J. 2021;9(8):929–37.
6 Dong J, Maj C, Tsavachidis S, Ostrom QT, Gharahkhani P, Anderson LA, et al. Sex-specific genetic associations for Barrett's esophagus and esophageal adenocarcinoma. Gastroenterology. 2020;159:2065–76.e1.
7 Buas MF, He Q, Johnson LG, Onstad L, Levine DM, Thrift AP, et al. Germline variation in inflammation-related pathways and risk of Barrett's oesophagus and oesophageal adenocarcinoma. Gut. 2017;66:1739–47.
8 Xie SH, Fang R, Huang M, Dai J, Thrift AP, Anderson LA, et al. Association between levels of sex hormones and risk of esophageal adenocarcinoma and Barrett's esophagus. Clin Gastroenterol Hepatol. 2020;18:2701–9.e3.
9 Quante M, Bhagat G, Abrams JA, Marache F, Good P, Lee MD, et al. Bile acid and inflammation activate gastric cardia stem cells in a mouse model of Barrett-like metaplasia. Cancer Cell. 2012;21:36–51.
10 Münch NS, Fang HY, Ingermann J, Maurer HC, Anand A, Kellner V, et al. High-fat diet accelerates carcinogenesis in a mouse model of Barrett's esophagus via interleukin 8 and alterations to the gut microbiome. Gastroenterology. 2019;157:492–506.e2.
11 Wani S, Rubenstein JH, Vieth M, Bergman J. Diagnosis and management of low-grade dysplasia in Barrett's esophagus: expert review from the clinical practice updates committee of the American Gastroenterological Association. Gastroenterology. 2016;151:822–35.
12 Duits LC, Phoa KN, Curvers WL, Ten Kate FJ, Meijer GA, Seldenrijk CA, et al. Barrett's oesophagus patients with low-grade dysplasia can be accurately risk-stratified after histological review by an expert pathology panel. Gut. 2015;64:700–6.
13 Duits LC, van der Wel MJ, Cotton CC, Phoa KN, Ten Kate FJW, Seldenrijk CA, et al. Patients with Barrett's esophagus and confirmed persistent low-grade dysplasia are at increased risk for progression to neoplasia. Gastroenterology. 2017;152:993–1001.e1.
14 Fitzgerald RC, di Pietro M, Ragunath K, Ang Y, Kang J-Y, Watson P. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut. 2014;63:7–42.
15 Snyder P, Dunbar K, Cipher DJ, Souza RF, Spechler SJ, Konda VJA. Aberrant p53 immunostaining in Barrett's esophagus predicts neoplastic progression: systematic review and meta-analyses. Dig Dis Sci. 2019;64:1089–97.
16 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. Gut. 2013;62:1676–83.
17 Stachler MD, Camarda ND, Deitrick C, Kim A, Agoston AT, Odze RD, et al. Detection of mutations in Barrett's esophagus before progression to high-grade dysplasia or adenocarcinoma. Gastroenterology. 2018;155:156–67.
18 van der Wel MJ, Coleman HG, Bergman JJ-GHM, Jansen M, Meijer SL; BOLERO working group. Histopathologist features predictive of diagnostic concordance at expert level among a large international sample of pathologists diagnosing Barrett's dysplasia using digital pathology. Gut. 2020;69:811–22.
19 Killcoyne S, Gregson E, Wedge DC, Woodcock DJ, Eldridge MD, de la Rue R, et al. Genomic copy number predicts esophageal cancer years before transformation. Nat Med. 2020;26:1726–32.
20 Contino G, Vaughan TL, Whiteman D, Fitzgerald RC. The evolving genomic landscape of Barrett's esophagus and esophageal adenocarcinoma. Gastroenterology. 2017;153:657–73.e1.
21 Nowicki-Osuch K, Zhuang L, Jammula S, Bleaney CW, Mahbubani KT, Devonshire G, et al. Molecular phenotyping reveals the identity of Barrett's esophagus and its malignant transition. Science. 2021;373(6556):760–7.
22 Kunze B, Wein F, Fang HY, Anand A, Baumeister T, Strangmann J, et al. Notch signaling mediates differentiation in Barrett's esophagus and promotes progression to adenocarcinoma. Gastroenterology. 2020;159:575–90.
23 Stachler MD, Taylor-Weiner A, Peng S, McKenna A, Agoston AT, Odze RD, et al. Paired exome analysis of Barrett's esophagus and adenocarcinoma. Nat Genet. 2015;47:1047–55.
24 The OCCAMS Consortium; Weaver JMJ, Ross-Innes CS, Shannon N, Lynch AG, Forshew T, et al. Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis. Nat Genet. 2014;46:837–43.

25 Buas MF, Levine DM, Makar KW, Utsugi H, Onstad L, Li X, et al. Integrative post-genome-wide association analysis of CDKN2A and TP53 SNPs and risk of esophageal adenocarcinoma. Carcinogenesis. 2014;35:2740–7.
26 Maley CC, Galipeau PC, Finley JC, Wongsurawat VJ, Li X, Sanchez CA, et al. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. Nat Genet. 2006;38:468–73.
27 Reid BJ, Haggitt RC, Rubin CE, Rabinovitch PS. Barrett's esophagus. Correlation between flow cytometry and histology in detection of patients at risk for adenocarcinoma. Gastroenterology. 1987;93:1–11.
28 Hadjinicolaou AV, van Munster SN, Achilleos A, Santiago Garcia J, Killcoyne S, Ragunath K, et al. Aneuploidy in targeted endoscopic biopsies outperforms other tissue biomarkers in the prediction of histologic progression of Barrett's oesophagus: a multicentre prospective cohort study. EBioMedicine. 2020;56:102765.
29 Fritcher EGB, Brankley SM, Kipp BR, Voss JS, Campion MB, Morrison LE, et al. A comparison of conventional cytology, DNA ploidy analysis, and fluorescence in situ hybridization for the detection of dysplasia and adenocarcinoma in patients with Barrett's esophagus. Hum Pathol. 2008;39:1128–35.
30 Timmer MR, Martinez P, Lau CT, Westra WM, Calpe S, Rygiel AM, et al. Derivation of genetic biomarkers for cancer risk stratification in Barrett's oesophagus: a prospective cohort study. Gut. 2016;65:1602–10.
31 Bajpai M, Panda A, Birudaraju K, Van Gurp J, Chak A, Das KM, et al. Recurring translocations in Barrett's esophageal adenocarcinoma. Front Genet. 2021;12:674741.
32 Eads CA, Lord RV, Wickramasinghe K, Long TI, Kurumboor SK, Bernstein L, et al. Epigenetic patterns in the progression of esophageal adenocarcinoma. Cancer Res. 2001;61:3410–8.
33 Kaz AM, Grady WM, Stachler MD, Bass AJ. Genetic and epigenetic alterations in Barrett's esophagus and esophageal adenocarcinoma. Gastroenterol Clin North Am. 2015;44:473–89.
34 Jin Z, Cheng Y, Gu W, Zheng Y, Sato F, Mori Y, et al. A multicenter, double-blinded validation study of methylation biomarkers for progression prediction in Barrett's esophagus. Cancer Res. 2009;69:4112–5.
35 Alvi MA, Liu X, O'Donovan M, Newton R, Wernisch L, Shannon NB, et al. DNA methylation as an adjunct to histopathology to detect prevalent, inconspicuous dysplasia and early-stage neoplasia in Barrett's esophagus. Clin Cancer Res. 2013;19:878–88.
36 Moinova HR, LaFramboise T, Lutterbaugh JD, Chandar AK, Dumot J, Faulx A, et al. Identifying DNA methylation biomarkers for non-endoscopic detection of Barrett's esophagus. Sci Transl Med. 2018;10:eaao5848.
37 Redston M, Noffsinger A, Kim A, Akarca FG, Rara M, Stapleton D, et al. Abnormal TP53 predicts risk of progression in patients with Barrett's esophagus regardless of a diagnosis of dysplasia. Gastroenterology. 2021. Epub ahead of print.
38 Bird-Lieberman EL, Dunn JM, Coleman HG, Lao-Sirieix P, Oukrif D, Moore CE, et al. Population-based study reveals new risk-stratification biomarker panel for Barrett's esophagus. Gastroenterology. 2012;143:927–35.e3.
39 Davison JM, Goldblum J, Grewal US, McGrath K, Fasanella K, Deitrick C, et al. Independent blinded validation of a tissue systems pathology test to predict progression in patients with Barrett's esophagus. Am J Gastroenterol. 2020;115:843–52.
40 Frei NF, Khoshiwal AM, Konte K, Bossart EA, Stebbins K, Zhang Y, et al. Tissue systems pathology test objectively risk stratifies Barrett's esophagus patients with low-grade dysplasia. Am J Gastroenterol. 2021;116:675–82.
41 Frei NF, Konte K, Bossart EA, Stebbins K, Zhang Y, Pouw RE, et al. Independent validation of a tissue systems pathology assay to predict future progression in nondysplastic Barrett's esophagus: a spatial-temporal analysis. Clin Transl Gastroenterol. 2020;11:e00244.
42 Li X, Galipeau PC, Paulson TG, Sanchez CA, Arnaudo J, Liu K, et al. Temporal and spatial evolution of somatic chromosomal alterations: a case-cohort study of Barrett's esophagus. Cancer Prev Res. 2014;7:114–27.
43 Li X, Paulson TG, Galipeau PC, Sanchez CA, Liu K, Kuhner MK, et al. Assessment of esophageal adenocarcinoma risk using somatic chromosome alterations in longitudinal samples in Barrett's esophagus. Cancer Prev Res. 2015;8:845–56.
44 Codipilly DC, Krishna Chandar A, Wang KK, Katzka DA, Goldblum JR, Thota PN, et al. Wide-area transepithelial sampling for dysplasia detection in Barrett's esophagus: a systematic review and meta-analysis. Gastrointest Endosc. 2022 Jan;95(1):51–9.e7.
45 Douville C, Moinova HR, Thota PN, Shaheen NJ, Iyer PG, Canto MI, et al. Massively parallel sequencing of esophageal brushings enables an aneuploidy-based classification of patients with Barrett's esophagus. Gastroenterology. 2021;160:2043–54.e2.
46 Ross-Innes CS, Chettouh H, Achilleos A, Galeano-Dalmau N, Debiram-Beecham I, MacRae S, et al. Risk stratification of Barrett's oesophagus using a non-endoscopic sampling method coupled with a biomarker panel: a cohort study. Lancet Gastroenterol Hepatol. 2017;2:23–31.