New models of neoplastic progression in Barrett’s oesophagus

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Abstract

Research in Barrett’s oesophagus, and neoplastic progression to OAC (oesophageal adenocarcinoma), is hobbled by the lack of good pre-clinical models that capture the evolutionary dynamics of Barrett’s cell populations. Current models trade off tractability for realism. Computational models are perhaps the most tractable and can be used both to interpret data and to develop intuitions and hypotheses for neoplastic progression. Tissue culture models include squamous cell lines, Barrett’s oesophagus cell lines and OAC cell lines, although it was recognized recently that BIC-1, SEG-1 and TE-7 are not true OAC cell lines. Some of the unrealistic aspects of the micro-environment in two-dimensional tissue culture may be overcome with the development of three-dimensional organotypic cultures of Barrett’s oesophagus. The most realistic, but least tractable, model is a canine surgical model that generates reflux and leads to an intestinal metaplasia. Alternatively, rat surgical models have gained popularity and should be tested for the common genetic features of Barrett’s oesophagus neoplastic progression in humans including loss of CDKN2A (cyclin-dependent kinase inhibitor 2A) and TP53 (tumour protein 53), generation of aneuploidy and realistic levels of genetic diversity. This last feature will be important for studying the effects of cancer-prevention interventions. In order to study the dynamics of progression and the effects of an experimental intervention, there is a need to follow animals longitudinally, with periodic endoscopic biopsies. This is now possible and represents an exciting opportunity for the future.

Introduction

Barrett’s oesophagus is an intestinal metaplasia of squamous oesophageal epithelium and is important clinically because it increases the chance of progression to OAC (oesophageal adenocarcinoma) 30–125-fold compared with people without Barrett’s oesophagus [1]. Injury from persistent gastroesophageal reflux is considered to be the causative agent, and treatment consists of medical therapy, aimed at lowering the frequency and acidity of reflux, and ablation. Unfortunately, even acid suppression by proton pump inhibitors does not usually induce regression of the Barrett’s metaplasia [2,3], and ablative procedures often fail to both completely eliminate all of the Barrett’s oesophagus tissue and to prevent recurrence of Barrett’s dysplasia [4]. Furthermore, there is evidence that Barrett’s oesophagus itself is an adaptation to acid reflux and may serve to protect the oesophagus from the development of life-threatening strictures and infections [5–7]. Since only 0.7% of people with Barrett’s oesophagus will progress to OAC each year [8], there is a critical need to distinguish patients at high risk of progression from low-risk patients, and to develop non-toxic cancer-prevention strategies. Both of these goals would be substantially aided by the development of good pre-clinical models of Barrett’s oesophagus.

Research during the last few decades has shown that neoplastic progression is not just a simple transition from normal to disease state, but a complex dynamic of clonal competition and evolution among somatic cells [9,10]. Thus cancer-prevention efforts are essentially efforts to affect and change the evolutionary dynamics of the pre-malignant cells. Because Barrett’s oesophagus can be followed longitudinally with multiple samples at each time point, Barrett’s oesophagus offers a unique opportunity for dissecting the evolutionary process of neoplastic progression, studying the impact of cancer-preventive interventions and extending those results to other cancers. The best pre-clinical models of Barrett’s oesophagus would reproduce the evolutionary dynamics of progression and the impact of interventions. Currently, there are no ideal pre-clinical models of Barrett’s oesophagus that capture the evolutionary dynamics of neoplastic progression. Those that exist range from the most tractable models, such as computational simulations and two-dimensional tissue culture, that lack many of the significant details of the human disease in vivo, to animal models that are not physiologically similar to the human.

Key words: anastomosis, Barrett’s oesophagus, cancer prevention, disease model, neoplastic progression, oesophageal adenocarcinoma.

Abbreviations used: CDKN2A, cyclin-dependent kinase inhibitor 2A; hiHET, human telomerase reverse transcriptase; OAC, oesophageal adenocarcinoma; OGA, oesophagogastroduodenal anastomosis; TP53, tumour protein 53; TSP1, thrombospondin-1

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disease and require long periods of time to develop OAC. Important opportunities remain to better characterize our current pre-clinical models and to improve them.

**Computational models**

Computational models provide the ultimate level of control and information. Every detail of a computational model is by definition both available for observation and modification. Their main drawback is that many of the details of a biological system are not yet understood, so the representation of those details and dynamics in a computational model is in essence a hypothesis for what may be true of the biological system. In addition, representation of a biological system in a computer simulation requires the abstraction or exclusion of much of the complexity of the biology, and so the generality of the results of the models are always in question. Nevertheless, computational models have proved useful for the exploration of current theories, hypothesis generation, and the discovery of important holes in our understanding that are likely to be critical to the dynamics of the biological system. When one has to write down the details of the essential aspects of a biological system, one quickly realizes how little is known about that system.

Computational models can simulate the evolution of somatic cells over decades. We discovered that there is an important (and counterintuitive) interaction between the number of cancer genes (tumour-suppressor genes or oncogenes) that must be mutated in a single allele (dominant mutations) for the development of malignancy, and mutant lesions that increase the rate of (epi)genetic lesions [11]. The requirement of more mutations for the development of malignancy actually increased the chance of progression to malignancy, because they provided more opportunities for a mutator lesion to hitchhike on an expansion of the clone with a dominant cancer gene mutation. The generation of a large genetically unstable clone greatly increases the probability of progression in the model. This has been supported in a cohort of Barrett’s oesophagus patients in which the size of a clone with p53 LOH (loss of heterozygosity), aneuploidy or tetraploidy was significantly associated with progression to OAC [12].

Recently, we used a computational model to examine the dynamics of clonal expansion and found that clones on two-dimensional surfaces, like Barrett’s oesophagus, are likely to expand quadratically rather than exponentially [13]. This model fitted p53 mutant clone size data from a skin cancer mouse model better than an exponential model of clonal expansion. We also found that the shape of a clone differed depending on whether it had a proliferative fitness advantage or a survival fitness advantage. If the lesion driving the clonal expansion gave the clone a proliferative advantage over its neighbouring clones, then it tended to have a rougher, or concave, border (looked more ‘invasive’) than clones with a survival advantage, which produced a relatively smooth convex shape.

A variety of models have been developed to study the dynamics of cells within a crypt [14,15], which probably also apply to Barrett’s oesophagus. Most of these have been used to test the implications of alternative hypotheses for stem cell dynamics and differentiation, as well as lesions that may initiate carcinogenesis with uncontrolled growth [16–21]. Earlier models of stem cell dynamics were fitted to data on the conversion of polyclonal murine intestinal crypts into monoclonal crypts as well as crypt density and were used to infer crypt life-cycle dynamics [22–24].

**Tissue culture models**

**Squamous cell culture models**

Since Barrett’s oesophagus is hypothesized to originate from squamous epithelium, representative squamous cell lines could be of great value in research aimed at inducing transdifferentiation of squamous epithelium towards Barrett’s oesophagus. Using EPC2 cells, an oesophageal epithelial cell line transformed with hTERT (human telomerase reverse transcription), Kong et al. [28] showed that demethylation and CDX2 (Caudal-type homeobox 2) expression induced intestinalization. A weakness of this approach is the possibility that Barrett’s oesophagus does not derive from squamous cells.

**Barrett’s oesophagus cell culture models**

Palanca-Wessels et al. [26,27] described the derivation [26] and immortalization using an hTERT transfection [27] of four Barrett’s oesophagus cell lines. Three of those cell lines (CP-B, CP-C and CP-D) were derived from patients with high-grade dysplasia and exhibited lesions in both CDKN2A (cyclin-dependent kinase inhibitor 2A) (p16/INK4A) and TP53 (tumour protein 53) (p53), whereas the fourth cell line (CP-A), from a patient with only metaplastic Barrett’s oesophagus, also had inactivated CDKN2A, but is wild-type for TP53 [26], although it has extensive LOH on chromosome 5q, including APC (adenomatous polyposis coli). Another hTERT immortalized cell line (BAR-T) was described by Jaiswal et al. [28]. In contrast with the cell lines described by Palanca-Wessels et al. [26,27], this cell line initially showed both intact CDKN2A and TP53, and lost CDKN2A during adaptations to conditions in vitro [28]. Owing to functioning TP53, this cell line may be a good model of early Barrett’s oesophagus progression.

**OAC cell culture models**

A number of OAC cell lines have been described over the years [29–31]. However, recent research has called into question the identity of three of the most commonly used OAC cell lines (BIC-1, SEG-1 and TE-7) [29,32]. This caused a shortage of reliable DNA-fingerprinted OAC cell lines, although FLO and OE33 appear to be true OAC cell lines. Alvarez et al. [29] described the isolation of JH-EsoAd1, a cell line derived from moderately to poorly differentiated
OAC. Because DNA fingerprinting confirmed its identity, JH-EsoAd1 also holds promise for future research in OAC.

**Organotypic models**

One of the major drawbacks of two-dimensional tissue culture models is that they fail to represent the micro-environment of the tumour, which has been shown to be important in progression [33]. In order to mimic aspects of the tumour micro-environment, organotypic models of oesophageal keratinocytes have been developed [34,35]. In these models, the epithelial cells are cultured on top of a layer of collagen and fibroblasts, with medium being fed into the system from below. Such organotypic models have been used to study both squamous cell carcinoma [34] and Barrett’s oesophagus [35] development. Stairs et al. [35] found that co-expression of c-myc and CDX1 of EPC2-hTERT cells in an organotypic model induced early Barrett’s oesophagus characteristics such as KRT8 (cytokeratin 8) and MUC5 (mucin 5) expression [35]. Organotypic models offer more realistic conditions for studying neoplastic progression than two-dimensional models, and the cells can remain viable and proliferative for more than 3 weeks without passing. However, they typically lack important aspects of the micro-environment of Barrett’s oesophagus, including inflammatory cells and endothelial cells. Furthermore, they do not form into crypts, and so lack realistic stem cell and differentiation dynamics. Recent work on LGR5 (leucine-rich-repet-containing G-protein-coupled receptor 5)-positive stem cells in colon crypts has shown that crypts can be induced to develop in a three-dimensional spheroid model [36]. This may provide an alternative approach for developing organotypic models of Barrett’s oesophagus.

**Animal models**

The most popular animals models of Barrett’s oesophagus are rat surgical models. There are also some mouse models and an older, but perhaps more physiologically realistic, canine model.

**Rat models**

There are a variety of rat surgical models, some involving a gastrectomy, and often an anastomosis between the jejunum or duodenum and the oesophagus [37,38]. In some cases, these models produce more Barrett’s oesophagus or OAC with the addition of iron [39]. Perhaps the most promising animal model of Barrett’s oesophagus is the OGDA (oesophagogastroduodenal anastamosis) model [40–42]. After 40 weeks, 25.6% of the animals develop OAC [41]. The OGDA model is particularly intriguing in that the contents of the duodenum are no longer acidic, and so this model shows that bile reflux is sufficient to generate both Barrett’s oesophagus and OAC. This result questions the emphasis on acid reflux and acid suppression in the treatment of Barrett’s oesophagus and the prevention of OAC.

**Dog model**

One of the earliest animal models of Barrett’s oesophagus was a canine model which involved surgical removal of the mucosa of the distal oesophagus as well as generation of a hiatal hernia and cardioplasty, which induced columnar epithelium after 8 weeks [37]. This is perhaps the most realistic model of Barrett’s oesophagus since it is driven by reflux and wounding. Experiments with this model, leaving a barrier of squamous cells between the stomach and denuded region of the oesophagus, suggested that Barrett’s oesophagus does not develop by migration of columnar gastric epithelium [46] and that islands of columnar cells seem to develop initially around submucosal glands [47]. Unfortunately, the canine model of Barrett’s oesophagus seems to have fallen out of use.

**Mouse models**

There has been some attempt to develop mouse surgical models, similar to the rat model, including an oesophago-jejunostomy model with N-methyl-N-benzylnitrosamine, which resulted in 37% of the mice developing OAC [44]. However, this model appears to be more difficult to implement than the rat model and has not been as popular.

Genetic models have often been used to study the genetic underpinnings of carcinogenesis. There are currently no commonly used genetic models of Barrett’s oesophagus in mice. However, one team has reported the development of intestinal metaplasia of the oesophagus in mice defective for TSP1 (thrombospondin-1) [45]. The wide availability of tools and reagents for manipulating mouse models makes this an attractive avenue for research. Unfortunately, the apparent development of Barrett’s oesophagus in the TSP1 mouse model was but one of many observed phenotypes, which did not develop in all cases and did not progress to OAC in the period of study.

Both the mouse and rat gastrointestinal tract have significant differences from the human gastrointestinal tract. In particular, rats and mice have a squamous fore-stomach which may result in different dynamics of the development of intestinal metaplasia in the oesophagus compared with humans. Furthermore, the inactivation of a gene throughout the entire organism, or even an entire organ, poorly represents the process of somatic evolution where a gene is inactivated in a single cell and whether or not that clone expands depends on the fitness effects of the inactivated gene on the clone, as well as stochastic dynamics of cell turnover in the tissue.
The ideal model

An ideal model of Barrett’s oesophagus would capture the dynamics of progression from normal squamous epithelium to OAC. This would include a transition from squamous to Barrett’s oesophagus and would involve expansions of clones with lesions in CDKN2A as well as TP53 and the development of tetraploidy and aneuploidy late in progression [43]. It seems likely that inclusion of the chronic inflammation observed in the human condition will be necessary for a model to replicate the dynamics of the human disease, although this has not yet been shown. Importantly, an ideal model would allow for longitudinal sampling within the same organism so that progression to OAC could be tracked over time. In addition, the model should allow for multiple samples at any one time point so that clonal expansions could be observed. It would be helpful if progression to OAC was both reliable and rapid, although accelerated progression may be incompatible with realistic evolutionary dynamics of progression. Finally, the genetic diversity of human Barrett’s oesophagus should be captured in the model so that realistic responses to interventions may be modelled. It may be the case that the OGDA rat model or the dog model may fit all (or most) of these criteria. Genetic characterization of the animal models will be important for their validation.

Major open questions

Good pre-clinical models of Barrett’s oesophagus would facilitate the investigation of a host of important questions in the field. A basic question in cancer biology for any tumour is to determine the sets of (epi)genetic lesions that are both necessary and sufficient to generate a malignancy in sporadic cancers. Genome-wide analyses of longitudinal samples from the same animal or patient would facilitate the discovery of potential lesions that could then be tested by introducing them sequentially into the Barrett’s oesophagus cells of an animal model. Observational studies, using genetic or epigenetic markers in multiple biopsies from each time point of a longitudinal study, could also be used to measure the frequency of clonal expansions during neoplastic progression. There is considerable disagreement in the literature over the frequency of such expansions (e.g. 1 compared with 20) [48,49], and very little data to resolve the debate. Associating particular lesions with those clonal expansions would also help to determine which of the necessary lesions in neoplastic progression increase the fitness of a clone, and which are evolutionarily neutral or deleterious. Lesions that cause clonal expansions make for good biomarkers that can characterize the somatic evolution that drives progression and the effects of cancer-preventive interventions.

Because neoplastic progression is a process of somatic evolution, cancer prevention is fundamentally an attempt to have an impact on and control that evolutionary process. However, the evolutionary effects of our interventions are virtually unstudied. A critical question remains what (epi)genetic lesions are selected by an intervention? That is, what (epi)genetic lesions provide a relative fitness advantage in the altered micro-environment induced by the intervention? Some of those lesions may generate resistance to the cancer-preventive intervention, and an understanding of the mechanism of that resistance should help us to identify patients unlikely to benefit from the intervention and to develop second line or multidrug cancer-prevention treatments that can overcome the evolution of resistance [9,52]. One approach to cancer prevention would be to alter the fitness of oesophageal squamous cells relative to Barrett’s oesophagus cells, so that the squamous epithelium could out-compete and replace the Barrett’s oesophagus tissue. This might be done by the traditional approach of reducing the fitness of Barrett’s oesophagus cells below the fitness of squamous cells or by increasing the fitness of squamous cells above that of Barrett’s oesophagus cells [53].

Conclusions

Current pre-clinical models of Barrett’s oesophagus range from the most tractable and least realistic computational and tissue culture models to a set of physiologically unrealistic animal models (with the possible exception of the canine model). Some of these models show promise, but will require further genetic and epigenetic characterization to validate their utility for studying the human disease. Important opportunities remain for both improving upon current models and the development of new models of Barrett’s oesophagus. Such pre-clinical models hold the promise of advancing both our knowledge and management of Barrett’s oesophagus, but will only be truly effective if they are used in longitudinal studies that can characterize the somatic evolution that drives progression and the effects of cancer-preventive interventions.

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