Published in final edited form as:
Biochem Soc Trans. 2010 April ; 38(2): 331–336. doi:10.1042/BST0380331.

New Models of Neoplastic Progression in Barrett's Esophagus

Kirill Pavlov[1] and **Carlo C. Maley**[2]
Carlo C. Maley: cmaley@alum.mit.edu

[1] University Medical Center Groningen, Division of Surgical Oncology, Department of Surgery, University Medical Center Groningen, University of Groningen, Hanzeplein 1 9700 RB, Groningen, The Netherlands [2] Molecular and Cellular Oncogenesis Program, The Wistar Institute, 3601 Spruce St. Philadelphia, PA, 19104, U.S.A. Genomics and Computational Biology Graduate Program and the Cell and Molecular Biology Graduate Program, University of Pennsylvania, phone: 215-495-6838

Abstract

Research in Barrett's oesophagus (BO), and neoplastic progression to oesophageal adenocarcinoma (OAC), is hobbled by the lack of good preclinical 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, BO cell lines and OAC cell lines, though it was recently recognized that BIC-1, SEG-1 and TE-7 are not true OAC cell lines. Some of the unrealistic aspects of the microenvironment in two-dimensional tissue culture may be overcome with the development of three-dimensional organotypic cultures of BO. 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 BO neoplastic progression in humans including loss of CDKN2A, TP53, 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 esophagus (BE) is an intestinal metaplasia of squamous esophageal epithelium, and is important clinically because it increases the chance of progression to esophageal adenocarcinoma (EA) by 30 to 125-fold over people without BE (1). Injury from persistent gastric-duodenal reflux is considered 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 Barrett's tissue and to prevent recurrence of Barrett's dysplasia (4). Furthermore, there is evidence that BE itself is an adaptation to acid reflux and may serve to protect the esophagus from the development of life-threatening strictures and infections (5–7). Since only 0.7% of people with BE will progress to EA per year (8), there is a critical need to distinguish patients at high risk for 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 BE.

Research during the last few decades have 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 impact and change the evolutionary dynamics of the pre-malignant cells. Because BE can be followed longitudinally with multiple samples at each time point, BE offers a unique opportunity for dissecting the evolutionary process of neoplastic progression, studying the impact of cancer prevention interventions and generalizing those results to other cancers. The best pre-clinical models of BE would recapitulate the evolutionary dynamics of progression and the impact of interventions.

Currently, there are no ideal pre-clinical models of BE 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 disease and require long periods of time to develop EA. 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 computation 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 (tumor suppressor genes or oncogenes) that must be mutated in a single allele (dominant mutations), for the development of malignancy, and mutator 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 BE patients in which the size of a clone with p53 loss of heterozygosity (LOH), aneuploidy or tetraploidy was significantly associated with progression to EA (12).

Recently, we used a computational model to examine the dynamics of clonal expansion and found that clones on two-dimensional surfaces, like BE, are likely to expand quadratically rather than exponentially (13). This model fit 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 neighboring 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 likely also apply to BE. 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 fit to data on the conversion of polyclonal murine intestinal crypts to monoclonal crypts as well as crypt density and used to infer crypt lifecycle dynamics (22–24).

Tissue Culture Models

Squamous cell culture models

Since BE 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 BE. Using EPC2 cells, an esophageal epithelial cell line transformed with hTERT, Kong et al. showed that demethylation and CDX2 expression induced intestinalization (25). A weakness of this approach is the possibility that BE does not derive from squamous cells.

Barrett's Esophagus cell culture models

Palanca-Wessels et al. described the derivation (26) and immortalization using an hTERT transfection (27) of four BE 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 (p16/INK4A) and TP53 (p53), while the fourth cell line (CP-A), from a patient with only metaplastic BE, also has inactivated CDKN2A but is wildtype for TP53 (26), though it has extensive LOH on chromosome 5q, including APC. Another hTERT immortalized cell line (BAR-T) was described by Jaiswal et al.(28). In contrast to the cell lines described by Palanca-Wessels et al., this cell line initially showed both intact CDKN2A and TP53, and lost CDKN2A during adaptations to conditions *in vitro* (28). Due to functioning TP53, this cell line maybe a good model of early BE progression.

Esophageal Adenocarcinoma cell culture models

A number of EA 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 EA cell lines (BIC-1, SEG-1 and TE-7) (29,32). This caused a shortage of reliable, DNA-fingerprinted EA cell lines, though FLO and OE33 appear to be true EA cell lines. Alvarez et al. (29) described the isolation of JH-EsoAd1, a cell line derived from moderately to poor differentiated EA. Because DNA fingerprinting confirmed its identity, JH-EsoAd1 also holds promise for future research in EA.

Organotypic Models

One of the major drawbacks of two-dimensional tissue culture models is that they fail to represent the microenvironment of the tumor, which has been shown to be important in progression (33). In order to mimic aspects of the tumor microenvironment, organotypic models of esophageal keratinocytes have been developed (34,35). In these models, the epithelial cells are cultured on top of a layer of collagen and fibroblasts, with media being fed into the system from below. Such organotypic models have been used to study both squamous cell carcinoma (34) and BE (35) development. Stairs et al. found that co-expression of c-myc and CDX1 of EPC2-hTERT cells in an organotypic model induced early BE-characteristics such as cytokeratin 8 and MUC5 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 three weeks, without passaging. However, they typically lack important aspects of the microenvironment of BE, 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 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 BE.

Animal Models

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

Rat models

There are a variety of surgical rat models, some involving a gastrectomy, and often an anastamosis between the jejunum or duodenum and the esophagus (37,38). IN some cases, these models produce more BE or EA with the addition of iron (39). Perhaps the most promising animal model of BE is the esophagogastroduodenal anastamosis (EDGA) model (40–42). After 40 weeks, 25.6% of the animals develop EA (41). The EDGA 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 BE and EA. This result questions the emphasis on acid reflux and acid suppression in the treatment of BE and the prevention of EA.

Important future questions for the EDGA model include whether or not the genetics of BE and progression to EA in this model match those observed in the human condition, such as the inactivation of CDKN2A (p16) and TP53 (p53), as well as the development of aneuploidy and tetraploidy (43). The large size of the rat esophagus, and the feasibility of endoscopic biopsy in this model suggest that the EDGA rats could be followed longitudinally with serial sampling to assess the genetic of progression in this model.

Mouse models

There has been some attempt to develop mouse surgical models, similar to the rat model, including an esophagojejunostomy model with *N*-methyl-*N*-benzylnitrosamine, which resulted in 37% of the mice developing EA (44). However this model appears to be harder 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 BE in mice. However, one team has reported the development of intestinal metaplasia of the esophagus in mice defective for thrombospondin-1 (TSP1) (45). The wide availability of tools and reagents for manipulating mouse models makes this an attractive avenue for research. Unfortunately, the apparent development of BE in the TSP1 mouse model was but one of many observed phenotypes, which did not develop in all cases, and did not progress to EA in the period of study. Both the mouse and rat gastrointestinal tract have significant differences from the human GI tract. In particular, rats and mice have a squamous fore-stomache which may result in different dynamics of the development of intestinal metaplasia in the esophagus compared to 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.

Dog model

One of the earliest animal models of BE was a canine model which included surgical removal of the mucosa of the distal esophagus 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 BE 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 eosphagus, suggested that BE does not develop by migration of columnar gastric epithelium (46) and that islands of columnar cells seem to initially develop around submucosal glands (47). Unfortunately, the canine model of BE seems to have fallen out of use.

The Ideal Model

An ideal model of BE would capture the dynamics of progression from normal squamous epithelium to EA. This would include a transition from squamous to BE 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, though this has not yet been shown. Importantly, an ideal model would allow for longitudinal sampling within the same organism so that progression to EA 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 EA was both reliable and rapid, though accelerated progression may be incompatible with realistic evolutionary dynamics of progression. Finally, the genetic diversity of human BE should be captured in the model so that realistic responses to interventions may be modeled. It may be the case that the EGDA 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 BE would facilitate the investigation of a host of important questions in the field. A basic question in cancer biology for any tumor 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 BE cells of an animal model. Obsevational 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 vs. 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 be detected with few samples and can dramatically increase the probability of further progression (11,12).

Because clonal expansions dramatically increase the probability of progression to malignancy and can lead to secondary cancers, the inhibition of clonal expansions represents an attractive approach to cancer prevention. A first, critical step in such efforts will be to discover the mechanism of such expansions. Does a clone expand by crypt bifurcation/budding, and if so do the new crypts replace their neighbors by some mechanism, or just generate more densly packed crypts? Alternatively, clones may spread by surviving the wounding effects of reflux and then repopulating the denuded regions (13,50). In addition, local “metastases” of stem cells within a tissue have been hypothesized to spread a clone (51).

Because neoplastic progression is a process of somatic evolution, cancer prevention is fundamentally an attempt to impact 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 microenvironment induced by the intervention? Some of those lesions may generate resistance to the cancer prevention 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 manage the evolution of resistance (9,52). One approach to cancer prevention would be to alter the fitness of esophageal squamous cells relative to BE cells, so that the squamous epithelium could out-compete and replace the BE tissue. This might be done by the traditional approach of reducing the fitness of BE cells below the fitness of squamous cells, or by increasing the fitness of squamous cells above that of BE cells (53).

Conclusions

Current pre-clinical models of BE 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 BE. Such pre-clinical models hold the promise of advancing both our knowledge and management of BE, 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.

Acknowledgments

This work was supported in part by the Landon AACR Innovator Award for Cancer Prevention, and NIH grants R03 CA137811, P01 CA91955, P30 CA010815, R01 CA119224 and R01 CA140657.

References

1. Haggitt RC. Barrett’s esophagus, dysplasia, and adenocarcinoma. Hum Pathol 1994;25:982–93. [PubMed: 7927321]
2. Jankowski JA, Anderson M. Review article: management of oesophageal adenocarcinoma -- control of acid, bile and inflammation in intervention strategies for Barrett’s oesophagus. Aliment Pharmacol Ther 2004;20 (Suppl 5):71–80. [PubMed: 15456468]
3. Leedham S, Jankowski J. The evidence base of proton pump inhibitor chemopreventative agents in Barrett’s esophagus--the good, the bad, and the flawed! Am J Gastroenterol 2007;102:21–3. [PubMed: 17266685]
4. Adler DC, Zhou C, Tsai TH, et al. Three-dimensional optical coherence tomography of Barrett’s esophagus and buried glands beneath neosquamous epithelium following radiofrequency ablation. Endoscopy 2009;41:773–6. [PubMed: 19746317]
5. Orlando, RC. Mucosal Defense in Barrett’s Esophagus. In: Sharma, P., editor. Barrett’s Esophagus and Esophageal Adenocarcinoma. Oxford, UK: Blackwell Publishing, Ltd; 2006. p. 60-72.
6. Jovov B, Van Itallie CM, Shaheen NJ, et al. Claudin-18: a dominant tight junction protein in Barrett’s esophagus and likely contributor to its acid resistance. Am J Physiol Gastrointest Liver Physiol 2007;293:G1106–13. [PubMed: 17932229]
7. Tobey NA, Argote CM, Vanegas XC, Barlow W, Orlando RC. Electrical parameters and ion species for active transport in human esophageal stratified squamous epithelium and Barrett’s specialized columnar epithelium. Am J Physiol Gastrointest Liver Physiol 2007;293:G264–70. [PubMed: 17431220]
8. Thomas T, Abrams KR, De Caestecker JS, Robinson RJ. Meta analysis: Cancer risk in Barrett’s oesophagus. Aliment Pharmacol Ther 2007;26:1465–77. [PubMed: 17900269]

9. Merlo LM, Pepper JW, Reid BJ, Maley CC. Cancer as an evolutionary and ecological process. Nat Rev Cancer 2006;6:924–35. [PubMed: 17109012]
10. Pepper J, Scott Findlay C, Kassen R, Spencer S, Maley C. Cancer research meets evolutionary biology. Evolutionary Applications 2009;2:62–70.
11. Maley CC, Forrest S. Exploring the relationship between neutral and selective mutations in cancer. Artificial Life 2000;6:325–45. [PubMed: 11348585]
12. Maley CC, Galipeau PC, Li X, et al. The combination of genetic instability and clonal expansion predicts progression to esophageal adenocarcinoma. Cancer Res 2004;64:7629–33. [PubMed: 15492292]
13. Chao DL, Eck JT, Brash DE, Maley CC, Luebeck EG. Preneoplastic lesion growth driven by the death of adjacent normal stem cells. Proc Natl Acad Sci U S A 2008;105:15034–9. [PubMed: 18815380]
14. van Leeuwen IM, Byrne HM, Jensen OE, King JR. Crypt dynamics and colorectal cancer: advances in mathematical modelling. Cell Prolif 2006;39:157–81. [PubMed: 16671995]
15. van Leeuwen IM, Edwards CM, Ilyas M, Byrne HM. Towards a multiscale model of colorectal cancer. World J Gastroenterol 2007;13:1399–407. [PubMed: 17457972]
16. Pepper JW, Sprouffske K, Maley CC. Animal Cell Differentiation Patterns Suppress Somatic Evolution. PLoS Comput Biol 2007;3:e250. [PubMed: 18085819]
17. Loeffler M, Roeder I. Conceptual models to understand tissue stem cell organization. Curr Opin Hematol 2004;11:81–7. [PubMed: 15257023]
18. Johnston MD, Edwards CM, Bodmer WF, Maini PK, Chapman SJ. Mathematical modeling of cell population dynamics in the colonic crypt and in colorectal cancer. Proc Natl Acad Sci U S A 2007;104:4008–13. [PubMed: 17360468]
19. d'Onofrio A, Tomlinson IP. A nonlinear mathematical model of cell turnover, differentiation and tumorigenesis in the intestinal crypt. J Theor Biol 2007;244:367–74. [PubMed: 17049944]
20. Boman BM, Fields JZ, Cavanaugh KL, Guetter A, Runquist OA. How dysregulated colonic crypt dynamics cause stem cell overpopulation and initiate colon cancer. Cancer Res 2008;68:3304–13. [PubMed: 18451157]
21. Meineke FA, Potten CS, Loeffler M. Cell migration and organization in the intestinal crypt using a lattice-free model. Cell Prolif 2001;34:253–66. [PubMed: 11529883]
22. Loeffler M, Birke A, Winton D, Potten C. Somatic mutation, monoclonality and stochastic models of stem cell organization in the intestinal crypt. J Theor Biol 1993;160:471–91. [PubMed: 8501919]
23. Loeffler M, Bratke T, Paulus U, Li YQ, Potten CS. Clonality and life cycles of intestinal crypts explained by a state dependent stochastic model of epithelial stem cell organization. J Theor Biol 1997;186:41–54. [PubMed: 9176636]
24. Totafurno J, Bjerknes M, Cheng H. The crypt cycle. Crypt and villus production in the adult intestinal epithelium. Biophys J 1987;52:279–94. [PubMed: 3663832]
25. Kong J, Nakagawa H, Isariyawongse BK, et al. Induction of intestinalization in human esophageal keratinocytes is a multistep process. Carcinogenesis 2009;30:122–30. [PubMed: 18845559]
26. Palanca-Wessels MC, Barrett MT, Galipeau PC, Rohrer KL, Reid BJ, Rabinovitch PS. Genetic analysis of long-term Barrett's esophagus epithelial cultures exhibiting cytogenetic and ploidy abnormalities. Gastroenterology 1998;114:295–304. [PubMed: 9453489]
27. Palanca-Wessels MC, Klingelhutz A, Reid BJ, et al. Extended lifespan of Barrett's esophagus epithelium transduced with the human telomerase catalytic subunit: a useful in vitro model. Carcinogenesis 2003;24:1183–90. [PubMed: 12807723]
28. Jaiswal KR, Morales CP, Feagins LA, et al. Characterization of telomerase-immortalized, non-neoplastic, human Barrett's cell line (BAR-T). DisEsophagus 2007;20:256–64.
29. Alvarez H, Koorstra JB, Hong SM, et al. Establishment and characterization of a bona fide barrett esophagus-associated adenocarcinoma cell line. Cancer BiolTher 2008:7.
30. Soldes OS, Kuick RD, Thompson IA, et al. Differential expression of Hsp27 in normal oesophagus, Barrett's metaplasia and oesophageal adenocarcinomas. BrJCancer 1999;79:595–603.

31. Souza RF, Shewmake K, Beer DG, Cryer B, Spechler SJ. Selective inhibition of cyclooxygenase-2 suppresses growth and induces apoptosis in human esophageal adenocarcinoma cells. Cancer Res 2000;60:5767–72. [PubMed: 11059772]
32. Boonstra JJ, van der Velden AW, Beerens EC, et al. Mistaken identity of widely used esophageal adenocarcinoma cell line TE-7. Cancer Res 2007;67:7996–8001. [PubMed: 17804709]
33. Beacham DA, Cukierman E. Stromagenesis: the changing face of fibroblastic microenvironments during tumor progression. Semin Cancer Biol 2005;15:329–41. [PubMed: 15970443]
34. Okawa T, Michaylira CZ, Kalabis J, et al. The functional interplay between EGFR overexpression, hTERT activation, and p53 mutation in esophageal epithelial cells with activation of stromal fibroblasts induces tumor development, invasion, and differentiation. Genes Dev 2007;21:2788–803. [PubMed: 17974918]
35. Stairs DB, Nakagawa H, Klein-Szanto A, et al. Cdx1 and c-Myc foster the initiation of transdifferentiation of the normal esophageal squamous epithelium toward Barrett's esophagus. PLoSOne 2008;3:e3534.
36. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009
37. Koak Y, Winslet M. Changing role of in vivo models in columnar-lined lower esophagus. Dis Esophagus 2002;15:271–7. [PubMed: 12472470]
38. Li Y, Martin RC 2nd. Reflux injury of esophageal mucosa: experimental studies in animal models of esophagitis, Barrett's esophagus and esophageal adenocarcinoma. Dis Esophagus 2007;20:372–8. [PubMed: 17760649]
39. Goldstein SR, Yang GY, Chen X, Curtis SK, Yang CS. Studies of iron deposits, inducible nitric oxide synthase and nitrotyrosine in a rat model for esophageal adenocarcinoma. Carcinogenesis 1998;19:1445–9. [PubMed: 9744541]
40. Chen X, Wang S, Wu N, et al. Overexpression of 5-lipoxygenase in rat and human esophageal adenocarcinoma and inhibitory effects of zileuton and celecoxib on carcinogenesis. Clin Cancer Res 2004;10:6703–9. [PubMed: 15475461]
41. Chen X, Yang G, Ding WY, Bondoc F, Curtis SK, Yang CS. An esophagogastroduodenal anastomosis model for esophageal adenocarcinogenesis in rats and enhancement by iron overload. Carcinogenesis 1999;20:1801–8. [PubMed: 10469627]
42. Su Y, Chen X, Klein M, et al. Phenotype of columnar-lined esophagus in rats with esophagogastroduodenal anastomosis: similarity to human Barrett's esophagus. Lab Invest 2004;84:753–65. [PubMed: 15094711]
43. Galipeau PC, Li X, Blount PL, et al. NSAIDs modulate CDKN2A, TP53, and DNA content risk for progression to esophageal adenocarcinoma. PLoS Med 2007;4:e67. [PubMed: 17326708]
44. Xu X, LoCicero J 3rd, Macri E, Loda M, Ellis FH Jr. Barrett's esophagus and associated adenocarcinoma in a mouse surgical model. J Surg Res 2000;88:120–4. [PubMed: 10644476]
45. Crawford SE, Stellmach V, Murphy-Ullrich JE, et al. Thrombospondin-1 is a major activator of TGF-beta1 in vivo. Cell 1998;93:1159–70. [PubMed: 9657149]
46. Gillen P, Keeling P, Byrne PJ, West AB, Hennessy TP. Experimental columnar metaplasia in the canine oesophagus. Br J Surg 1988;75:113–5. [PubMed: 3349294]
47. Pollara WM, Cecconello I, Zilberstein B, Iria K, Pinotti HW. Regeneration of esophageal epithelium in the presence of gastroesophageal reflux. Arq Gastroenterol 1983;20:53–9. [PubMed: 6661093]
48. Meza R, Jeon J, Moolgavkar SH, Luebeck EG. Age-specific incidence of cancer: Phases, transitions, and biological implications. Proc Natl Acad Sci U S A 2008;105:16284–9. [PubMed: 18936480]
49. Beerenwinkel N, Antal T, Dingli D, et al. Genetic progression and the waiting time to cancer. PLoS Comput Biol 2007;3:e225. [PubMed: 17997597]
50. Zhang W, Hanks AN, Boucher K, et al. UVB-induced apoptosis drives clonal expansion during skin tumor development. Carcinogenesis 2005;26:249–57. [PubMed: 15498793]
51. van Oijen MG, Slootweg PJ. Oral field cancerization: carcinogen-induced independent events or micrometastatic deposits? Cancer Epidemiol Biomarkers Prev 2000;9:249–56. [PubMed: 10750662]
52. Potter JD. beta-Carotene and the role of intervention studies. Cancer Lett 1997;114:329–31. [PubMed: 9103323]

53. Maley CC, Reid BJ, Forrest S. Cancer prevention strategies that address the evolutionary dynamics of neoplastic cells: simulating benign cell boosters and selection for chemosensitivity. Cancer Epidemiol Biomarkers Prev 2004;13:1375–84. [PubMed: 15298961]