Chapter 1

Embryological signaling pathways in Barrett’s metaplasia development and malignant transformation; mechanisms and therapeutic opportunities

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Abstract

Barrett’s metaplasia of the esophagus (BE) is the precursor lesion of esophageal adenocarcinoma (EAC), a deadly disease with a 5-year overall survival of less than 20%. The molecular mechanisms of BE development and its transformation to EAC are poorly understood and current surveillance and treatment strategies are of limited efficacy. Increasing evidence suggests that aberrant signaling through pathways active in the embryological development of the esophagus contributes to BE development and progression to EAC. We discuss the role that the Bone Morphogenetic Protein, Hedgehog, Wingless-Type MMTV Integration Site Family (WNT) and Retinoic Acid signaling pathways play during embryological development of the esophagus and their contribution to BE development and malignant transformation. Modulation of these pathways provides new therapeutic opportunities. By integrating findings in developmental biology with those from translational research and clinical trials, this review provides a platform for future studies aimed at improving current management of BE and EAC.
Introduction

Barrett’s metaplasia of the esophagus (BE) is characterized by the presence of columnar epithelium in the distal part of the esophagus that is normally lined with squamous epithelium. The causative factors underlying the development of BE are debated, but persistent gastroesophageal reflux disease (GERD) is the main risk factor. The reported percentages of BE in GERD patients vary between 5 and 25% (1-4). BE is the single known precursor lesion of esophageal adenocarcinoma (EAC) and can progress through a multistep process from metaplastic to low-grade, then high-grade dysplasia and eventually Esophageal Adenocarcinoma (EAC) (5).

The management of BE and EAC is hampered by the limited effectiveness of surveillance strategies and of current therapeutic regimens in EAC treatment. While the presence of BE increases the risk of EAC development by more than 10-fold, recent large cohort studies estimate the annual progression rate at less than 0.5% (6-8). This low absolute rate of malignant transformation was the reason to question the relevance of current surveillance guidelines that recommend a gastroscopy every 3-5 years in BE patients (2,6,8,9). Despite some promising results of acid suppressant medication and non-steroidal anti-inflammatory drugs (NSAIDs), no pharmacological interventions have been proven effective in eradicating established BE or preventing its progression towards malignancy (10-14). Data from animal experiments and several cohort and case-control studies indicate that non-steroidal anti-inflammatory drugs (NSAIDs) may reduce the rate of BE progression towards malignancy and the risk of EAC (15-22). However, these findings are disputed by others (23) and the only available randomized controlled trial failed to show any benefit of Celecoxib in reducing progression towards malignancy (24). The therapeutic options for patients with EAC are also limited. Despite neoadjuvant chemoradiation and surgery the overall 5-year survival of EAC patients is less than 20%, highlighting the need for new therapeutic targets (25,26).

Aberrant activity of embryological signaling pathways is often implicated in the development of metaplasia and cancer. Experimental data suggests that signaling pathways active during esophageal development can provide a new perspective on BE and EAC development and thus provide potential novel therapeutic targets. The Bone Morphogenetic protein (BMP), Hedgehog (HH), Wingless-Type MMTV Integration Site Family (WNT) and Retinoic acid (RA) signaling pathways have a key role during embryological development of the esophagus and alterations in these pathways have been observed in both BE and EAC. In this review, we summarize the current knowledge regarding the role of BMP, WNT, HH and RA signaling pathways in esophageal embryology, their role in BE development and malignant transformation and discuss their potential as therapeutic targets in EAC treatment.
Signaling pathways in foregut embryology

The esophagus is derived from the embryological foregut. During embryological development the foregut lumen divides along the sagittal axis. The ventral half will become the trachea, lined with columnar epithelium while the dorsal half will become the esophagus, lined with squamous epithelium (27). Most of the understanding of the transcription factors and signaling pathways active in foregut separation and patterning comes from transgenic mouse models (Table 1). These models suggest that differentiation of foregut epithelium towards a squamous or columnar phenotype is regulated by the expression of three key transcription factors: NKX2.1, SOX2 and p63. NKX2.1 induces columnar differentiation of the foregut epithelium, while SOX2 and p63 expression is required for squamous differentiation. Knockout models showed that mice lacking NKX2.1 had impaired foregut separation and a common lumen lined with squamous epithelium (28), while in mice lacking SOX2 or p63 the esophagus was lined with columnar epithelium (28-30). Further experiments identified four main signaling pathways active in the embryological foregut: the BMP, HH, WNT and RA signaling pathways. These pathways are required for proper development of the trachea and esophagus and influence differentiation by regulating the expression of NKX2.1, SOX2 and p63 (31-34) (Figure 1).

Signaling through the BMP pathway contributes to a columnar differentiation of foregut epithelium. BMP ligands bind to type 1 and type 2 transmembrane BMP receptors (BMPR1 and BMPR2) that activate the SMAD transcription factors. The BMP4 ligand plays the most important role in foregut embryology. BMP4 is produced in the mesenchyme of the future tracheal domain where it stimulates columnar differentiation of foregut epithelium. High levels of ectopic BMP signaling in the esophageal foregut domain block the development of squamous epithelium and promote columnar differentiation (35), while abrogation of BMP signaling through inactivation of BMPR1 leads to ectopic expression of the squamous transcription factors SOX2 and p63 in the tracheal domain of the foregut (36). To prevent BMP-induced columnar differentiation of the esophageal foregut domain, BMP signaling in the esophageal foregut domain is repressed by the expression of Noggin, a BMP-antagonist. In mice with a homozygous deletion of the Noggin gene Nog the foregut fails to separate properly and the esophageal domain is covered with columnar epithelium, underlining the importance of proper spatial restriction of BMP signaling (37).

HH signaling is essential for proper foregut separation. Sonic Hedgehog (SHH) is one of three ligands of the HH signaling pathway. SHH binds to the membrane receptor Patched (PTCH). In an inactive state, PTCH represses Smoothened (SMO). Upon binding of SHH to PTCH, SMO repression is relieved and SMO activates the GLI transcription factors. Disrupted or absent HH signaling caused impaired foregut separation in mice (38-40), and alterations in downstream proteins of the HH signaling cascade such as GLI2,
GLI3 and FOXF1 yielded a similar phenotype (41,42). While HH signaling is essential for proper foregut separation, the effects of HH signaling on foregut differentiation are less clear. The esophageal domain of Shh-/- mice failed to develop squamous epithelium and was lined with columnar epithelium instead, suggesting that HH signaling contributes to the development of squamous epithelium (40). Moreover, SHH is present in normal adult squamous epithelium, where it stimulates proliferation of epithelial precursor cells (43). HH signaling is also a known inducer of the BMP pathway. During embryological development of the small intestine HH signaling induced BMP4 expression and was required for proper development of the columnar intestinal epithelium (44). Studies in mouse embryos showed that SHH is initially expressed in the tracheal domain, followed by a shift in expression to the esophageal domain (31,38). This ventral-dorsal shift is required for proper development of both trachea and esophagus, and a location-specific effect of SHH may explain the dual role of SHH with regard to the induction of squamous or columnar differentiation.
WNT signaling is important at various stages of embryonic development and contributes to a columnar differentiation of foregut epithelium. WNT ligands are secreted glycoproteins that bind to the Frizzled membrane receptors. Signaling induced by WNT ligands is classified as either canonical (beta-Catenin dependent) or non-canonical (beta-Catenin independent) (45,46) of which canonical WNT signaling is the best-studied signaling pathway in foregut embryology. Canonical WNT signaling was found to induce columnar differentiation of the tracheal foregut domain by inducing the expression of the columnar transcription factor NKX2.1 and repression of squamous transcription factors SOX2 and p63 (47). Mice models showed that abrogation of canonical WNT signaling through inactivation of beta-Catenin correlates with SOX2 expression in the tracheal foregut domain, while constitutive activation of WNT signaling via overexpression of beta-Catenin caused loss of SOX2 and reciprocal upregulation of NKX2.1 in the esophageal domain (48). One way in which WNT signaling could contribute to a columnar differentiation is through activation of BMP signaling. Deletion of the WNT ligands WNT2 and WNT2b in a mouse model reduced BMP4 signaling and caused loss of NKX2.1 in the tracheal foregut domain (47). This suggests that WNT signaling may be positioned upstream of BMP signaling in specifying the columnar differentiation of the tracheal foregut domain. Similarly to BMP-signaling, spatial restriction of WNT signaling in the foregut is important to prevent columnar differentiation of the esophageal foregut domain. During normal embryological development the esophageal foregut domain is shielded from WNT signaling by the expression of BARX1 (49). BARX1 is expressed in the mesoderm of the esophageal foregut domain where it stimulates the expression of the secreted Frizzle-related proteins 1 and 2 (sFRP1 and sFRP2). sFRPs are secreted polypeptides that prevent the binding of WNT ligands with the Frizzled receptor, and thereby antagonize WNT signaling (49,50).

RA signaling is essential for proper development of the esophagus and squamous differentiation. RA directs gene transcription by binding to nuclear Retinoic Acid Receptors (RAR-alpha, RAR-beta and RAR-gamma) and Retinoid X Receptors (RXR-alpha, RXR-beta and RXR-gamma) that act as transcription factors by binding to retinoic acid response elements of target genes. Knockout experiments generating $\text{Rar } \alpha^{-/}\beta^{-/}$ mice resulted in an undivided common foregut lumen lined with columnar epithelium (51). One way RA could contribute to a squamous differentiation of the foregut is by antagonizing HH and BMP signaling, since administration of RA inhibited HH and BMP signaling in hindgut development in mice (52). However, it is currently unknown whether a similar mechanism is active in foregut development.
Embryological signaling pathways in Barrett’s metaplasia development and progression towards malignancy

Besides having a role in the embryological foregut development, alterations in the BMP, WNT, HH and RA embryological signaling pathways have also been implicated in the development of BE and its progression towards EAC (Figure 2).

The BMP pathway is not active in normal squamous epithelium but is activated when squamous epithelium becomes inflamed due to GERD, even before the presence of detectable BE (53). Stromal BMP4 expression and activated SMAD proteins in squamous epithelium were observed in both human biopsies and animal models of BE (53,54). In *in vitro* experiments primary human esophageal cells treated with components of gastroesophageal refluxate showed an increase in BMP4 expression, suggesting additional paracrine BMP signaling (55). Also, incubation of primary human squamous cells with BMP4 induced a shift in the gene expression profile towards that of BE and expression of columnar cytokeratins CK7 and CK20 (53), suggesting that BMP4 contributes to a columnar
transdifferentiation of squamous esophageal cells. While the evidence implicating BMP signaling in the development of BE seems solid, the role of BMP signaling in the malignant transformation of BE requires further research. A single available study that looked at this found that a decrease in BMP4 protein expression in EAC compared to BE, but did not provide evidence of a corresponding decrease at mRNA level (56).

SHH expression in the normal esophagus is disputed, but it is likely that HH signaling contributes to BE development and subsequent malignant transformation. Studies reported either absent (57-60) or low-level SHH expression in the basal layer of the esophagus (43,61). However, expression of SHH and its receptor PTCH was increased in biopsies of both BE and EAC (60,62,63) and in a mouse model of BE (54,59,64). Also, an increased expression level of the GLI transcription factors was found in biopsies of EAC compared with biopsies of BE or squamous epithelium (65). HH signaling was found to contribute to BE development in two ways. First, HH signaling can activate the BMP4 promoter leading to BMP signaling (66). Second, HH signaling can induce epithelial SOX9 expression (67). SOX9 is a transcription factor associated with intestinal stem cells (68-70). Overexpression of SOX9 in an organotypic model of BE induced columnar differentiation of squamous cells and expression of the columnar cytokeratin CK8 (71). While data from patient material suggest that HH signaling is further increased during malignant transformation of BE, it is currently less clear how HH signaling contributes to the development of EAC. Possible explanations include a stimulatory effect of HH signaling on cell survival and proliferation (63,72), but this requires further study. The relationship between HH and BMP signaling is another area requiring further study. One study reported that BMP4 protein expression was lower in EAC compared to BE, but a corresponding decrease of mRNA was not found. This is remarkable, as HH signaling is an inducer of BMP signaling in embryonic development and BE development (44).

While WNT signaling does not appear to play a role in the development of BE, a progressive increase in WNT signaling is observed during malignant transformation of BE. Squamous epithelium lacks nuclear beta-Catenin indicating that canonical WNT signaling is not active (73,74). Refluxate components can activate WNT signaling in vitro (75,76) and WNT signaling activated SOX9 expression in the esophagus in a mouse model (77). However, nuclear beta-Catenin was not detected in patient biopsies of BE (73,77) suggesting that canonical WNT signaling is not involved in the development of BE metaplasia. In line with this notion, reflux increased the expression of the WNT-antagonist Dickkopf (78,79) that may prevent canonical WNT activation. Moreover, constitutive activation of beta-Catenin in normal squamous epithelium cultured in an organotypic model failed to induce a columnar phenotype. Instead, it yielded a thickened, hyperproliferative squamous epithelium (77,80), suggesting a role for WNT in regulating cellular proliferation. The fact that WNT signaling plays no role in the development of BE is remarkable, given its role
in inducing columnar differentiation during foregut development. On the other hand, WNT signaling does appear to be an important driver of BE malignant transformation. A progressive increase in WNT signaling (defined by nuclear beta-Catenin staining) was observed in biopsy samples of BE dysplasia and EAC (73,77,81) and this was confirmed in an EAC mouse model (82). Several mechanisms could contribute to WNT signaling activation during BE malignant transformation. Increased expression of the WNT2 ligand and hypermethylation of the promoter of the genes encoding WNT antagonists WNT Inhibitory Factor 1 (WIF1) and Secreted Frizzled-like Receptor Proteins were described in EAC (83-85), and promoter hypermethylation of the WIF1 gene in BE metaplasia was associated with progression towards EAC (84). Interestingly, while APC gene mutations are a common driver of colon cancer development (86), these mutations are rare (<5%) in EAC (87). In summary, the absence of activated canonical WNT signaling in BE suggests that WNT signaling is not involved in BE development. On the other hand, the progressive increase of WNT signaling in the metaplasia-dysplasia-carcinoma sequence may be a driving factor in the subsequent malignant transformation of BE.

During embryological development RA signaling contributes to a squamous differentiation of the esophageal foregut domain. In apparent contrast, RA biosynthesis is upregulated in BE (88,89). Incubation of human squamous epithelium biopsies with RA led to replacement of the squamous epithelium with a columnar, BE-like epithelium (89) and although RA failed to induce complete columnar differentiation in a squamous esophageal human cell line, it did increase the expression of MUC2, a glycoprotein typically expressed in BE but absent in SE (90). The contradictory effects of RA in esophageal embryology and BE development might be explained by differences in retinoic receptor subtype expression, which may lead to differences in response to RA. Compared to squamous epithelium RAR-alpha and RXR-gamma were upregulated and RAR-gamma and RXR-beta were downregulated in BE (91,92). Morphologically normal squamous epithelium from patients with BE and EAC already had alterations in retinoic acid receptor subtype expression (91,92). Given that only a minority of GERD patients develop BE (5%), pre-existing alterations in retinoic acid receptor subtype expression may predispose normal squamous epithelium to develop a metaplastic response after persistent GERD.

In contrast to increased RA signaling pathway activity in BE metaplasia, RA signaling is reduced in BE dysplasia and EAC (88). RA signaling seems to have a tumor suppressor effect, and RA administration inhibited cell proliferation in a variety of cancer cell lines (93-95) and induced apoptosis in a BE metaplasia cell line (96). Downregulation of the RAR-beta has been described in a variety of solid malignancies (97-99) including EAC (100). In addition, enhanced expression of the RA catabolizing enzyme CYP26A1 during the malignant transformation of BE has been linked to decreased RA levels in EAC, and overexpression of CYP26A1 in a BE dysplastic cell line promoted cell proliferation.
and survival by depletion of intracellular RA (88). Overall, RA signaling seems to have a dual role in BE; high levels of RA contribute to the development of BE but also prevent the development of EAC.

In summary, dysregulated signaling of the BMP, HH and RA pathways is associated with the development of BE metaplasia. During the progression of BE towards malignancy the SHH and WNT signaling pathways are upregulated while the RA and probably the BMP signaling pathways are downregulated.

**Therapeutic targeting of embryological signaling pathways in Barrett’s metaplasia and esophageal adenocarcinoma.**

Given the important roles of the BMP, HH, WNT and RA pathways in the development of BE metaplasia and progression towards EAC, therapeutic modulation of these pathways may be a valuable complimentary tool in the management of BE and EAC (Figure 2). Several possible therapeutic strategies are discussed below.

*Preventing Barrett’s metaplasia development*

The HH pathway can be targeted both at the level of the cell membrane receptors PTCH and SMO as well as more downstream at the GLI transcription factors (101,102). Preclinical studies suggest that inhibition of HH signaling might prevent the development of BE. Intraperitoneal injections of cyclopamine (a HH-antagonist) decreased gastric BMP4 expression in a mouse model (101). Another study used a surgical rat model of GERD in which rats treated with an oral SMO antagonist (Bristol-Meyers Squibb 833923) for 28 weeks had a significantly lower incidence of Barrett’s metaplasia (59% versus 95% in the control group) (102). Since both animal groups underwent the same procedure to induce persistent GERD, blocking HH signaling can reduce the incidence of BE even in the continuous presence of GERD. The current lack of biomarkers capable of predicting which GERD patients will develop BE limits the options for chemoprevention in GERD patients. However, given the central role of SHH in BE development, modulating the HH-BMP axis might cause regression of established BE. Although this has not been tested, the HH-BMP axis is an attractive therapeutic target in BE.

*Blocking Barrett’s metaplasia progression towards malignancy*

The HH signaling pathway seems to play a role in the progression of BE towards dysplasia and EAC, and provides an attractive therapeutic target in the prevention of BE malignant transformation. While clinical studies are currently lacking, suppression of GLI in a rat model using a combination of ursodeoxycholic acid and aspirin significantly decreased the incidence of EAC (65).
WNT signaling is increased in BE dysplasia and EAC and presents a second therapeutic opportunity. Several clinically tested WNT antagonists are available, including NSAIDs. NSAIDs are known to downregulate WNT-signaling (103-106) and were found to decrease the incidence of several epithelial cancers including colon, gastric and breast (107,108). It is currently unclear whether NSAID can prevent EAC development in patients with BE. NSAIDs prevented malignant transformation of BE in cohort studies (15), but the single reported randomized trial failed to find any chemopreventive effect of Celecoxib (24), despite the fact that Celecoxib inhibited WNT signaling in a colon cancer cell line (109). To clarify the potential value of NSAIDs in preventing EAC development an ongoing phase III trial (the AspECT trial) is investigating the effect of aspirin in combination with a proton pump inhibitor (esomeprazole) on progression towards malignancy (Table 2) (110).

RA signaling is a third potential signaling pathway that can be modulated to prevent malignant transformation of BE. The decrease of RA levels during malignant transformation suggests that RA may act as a tumor supressor. One explanation may be that RA signaling can antagonize canonical WNT signaling, possibly via competition with p300, a common co-activator factor for both RARs and beta-Catenin (111). The finding that RA levels decrease while canonical WNT signaling progressively increases during malignant transformation of BE suggests that RA may repress WNT signaling and thus prevent EAC development, but this remains to be tested.

While previous chemoprevention trials showed inconclusive results, this could be due to an incomplete coverage of the deregulated pathways. Targeting multiple deregulated signaling pathways by combining NSAIDs and HH antagonists with strategies to prevent a decrease in RA levels may thus translate into more effective chemoprevention strategies.

**Therapeutic targets in esophageal adenocarcinoma**

As described above, only limited evidence suggests that BMP4 is downregulated in EAC, and the exact mechanisms and clinical consequence of reduced BMP4 expression in EAC are still unclear. In colon cancer BMP4 induced differentiation and cell death of colorectal cancer stem cells and sensitized them to oxaliplatin and 5-fluorouracil, possibly as a result of WNT pathway inhibition (112-115). Several studies have suggested that a stem cell subpopulation exists in EAC (116,117), but thus far the effect of targeting the BMP pathway has not been studied in EAC.

HH signaling is upregulated in EAC. In vitro inhibition of the HH pathway with cyclopamine reduced tumor proliferation in EAC cell lines and caused regression of established cholangiocarcinoma tumors in a mouse xenograft model (72). A number of HH inhibitors are in development including small molecules targeting SMO (102,118) and blocking antibodies against the SHH ligand (72). In vitro these agents showed promising results by reducing cell viability (63,72). Several clinical trials are underway to evaluate...
their effects in various solid tumors as a single agent or in combination with chemotherapy (118-123) including one trial specifically evaluating the effect of an oral SMO antagonist (Bristol-Meyers Squibb 839323) in EAC (124) (Table 2). Furthermore, enhanced HH signaling was observed in patients with residual EAC tumors after chemoradiation, and blocking HH signaling augmented the efficacy of radiation in EAC cell lines (125), suggesting that modulation of HH signaling might also be used to augment the efficacy of current treatment modalities.

As mentioned earlier, canonical WNT signaling is deregulated in EAC. In vitro, restoration of the expression of WNT-antagonist WIF1 decreased cell proliferation and sensitized EAC cells to carboplatin treatment (84). Two ongoing phase II trials in EAC are investigating the added value of Celecoxib in neoadjuvant chemotherapy (126) or chemoradiation (127). In addition, several small molecule inhibitors of WNT signaling are undergoing phase I trials in solid tumors (128,129) (Table 2). Downregulation of WNT signaling by clinically available (NSAIDs) or experimental WNT-antagonists may increase the efficacy of current treatment strategies.

RA stimulates differentiation and limits cell proliferation in BE. EAC is characterized by low levels of RA (88,130,131) that may contribute to loss of differentiation and uncontrolled proliferation. Restoring RA levels could be an attractive therapeutic strategy in EAC. However, a small (13 EAC patients) phase II trial of RA and Interferon did not show any anti-tumor effect while being associated with considerable toxicity (132). An alternative strategy might be to use RA metabolism blocking agents (RAMBAs). RAMBAs counteract the elevated RA degradation and thus lead to increased RA levels. These agents increase tumor differentiation and apoptosis in vitro and reduce tumor growth in xenograft models of breast and prostate cancer (93,95). Moreover, whereas treatment with RA was associated with weight loss in the mouse models, RAMBAs were well tolerated in animal models and had no cytotoxic effect on normal, noncancerous cell lines (93-95).

**Conclusion and perspectives**

The increased understanding of embryological signaling pathways and their role in the development and malignant transformation of BE and EAC as outlined above suggests a number of novel therapeutic opportunities at all stages of the metaplasia-dysplasia-adenocarcinoma sequence. However, translation of the promising results obtained in preclinical studies into clinical applications faces several critical challenges. These include potential side effects from manipulating such fundamental pathways, difficulties in selective delivery of the drugs to the target tissue and the selection of patients that will benefit from treatment. Further research is required to address these issues and results from clinical studies testing the efficacy of these novel treatment strategies are eagerly awaited.
Acknowledgements

We would like to thank Dr. E.M E. van Straten for help with the figures. K. Pavlov is supported by a MD-PhD grant from the Junior Scientific Masterclass Groningen. This sponsor had no involvement in the preparation of this manuscript.
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Table 1: Pathways involved in foregut embryology and differentiation of the trachea and esophagus.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Gene</th>
<th>Protein function</th>
<th>Model</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMP</strong></td>
<td>Bmpr1a</td>
<td>Bone Morphogenetic Protein Receptor 1A</td>
<td>Mutant mice with constitutive BMP1 expression throughout the foregut</td>
<td>Clusters of simple columnar cells in the esophagus</td>
<td>[35]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mutant mice with Bmpr1 inactivation</td>
<td>Failure of foregut separation and p63+ Sox2+ positive cells in both the ventral and dorsal foregut domain.</td>
<td></td>
</tr>
<tr>
<td><strong>Nog</strong></td>
<td>Bmpr1a</td>
<td>BMP antagonist</td>
<td>Nog&lt;sup&gt;-/-&lt;/sup&gt; mice</td>
<td>Failure of proper foregut separation with tracheo-esophageal fistula Esophageal lining has characteristics of trachea</td>
<td>[37]</td>
</tr>
<tr>
<td><strong>SHH</strong></td>
<td>Shh</td>
<td>Signaling protein in hedgehog pathway</td>
<td>Shh&lt;sup&gt;-/-&lt;/sup&gt; mice</td>
<td>Dorsal foregut domain covered with columnar epithelium</td>
<td>[41]</td>
</tr>
<tr>
<td>Gli2</td>
<td></td>
<td>Transcription factors downstream of Shh.</td>
<td>Gli2&lt;sup&gt;+/+&lt;/sup&gt; Gli3&lt;sup&gt;-/-&lt;/sup&gt; and Gli2&lt;sup&gt;-/-&lt;/sup&gt; Gli3&lt;sup&gt;-/-&lt;/sup&gt; mice</td>
<td>Failure of foregut separation in Gli2&lt;sup&gt;+/+&lt;/sup&gt; Gli3&lt;sup&gt;-/-&lt;/sup&gt; mice</td>
<td>[31]</td>
</tr>
<tr>
<td>Gli3</td>
<td></td>
<td></td>
<td>Gli2&lt;sup&gt;-/-&lt;/sup&gt; Gli3&lt;sup&gt;-/-&lt;/sup&gt; mice</td>
<td>No esophagus or trachea development in Gli2&lt;sup&gt;-/-&lt;/sup&gt; Gli3&lt;sup&gt;-/-&lt;/sup&gt; mice</td>
<td></td>
</tr>
<tr>
<td>Foxf1</td>
<td></td>
<td>Transcription factor downstream of Shh.</td>
<td>Foxf1&lt;sup&gt;+/+&lt;/sup&gt; mice</td>
<td>Failure of foregut separation</td>
<td>[42]</td>
</tr>
<tr>
<td><strong>WNT</strong></td>
<td>Beta-catenin (encoded by the Ctnnb1 gene)</td>
<td>Central signal transducer in the canonical Wnt signaling pathway</td>
<td>Mutant mice with constitutive beta-catenin expression throughout the foregut</td>
<td>Loss of Nkx2.1 and expansion of Sox2 in the ventral foregut domain</td>
<td>[48]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mutant mice with inactivation of beta-catenin in the foregut</td>
<td>Loss of Sox2 and expansion of Nkx2.1 in the dorsal foregut domain</td>
<td></td>
</tr>
<tr>
<td>Wnt2/2b</td>
<td></td>
<td>Wnt ligand</td>
<td>Wnt2/2b&lt;sup&gt;+/+&lt;/sup&gt; mice</td>
<td>Reduced Bmp4 signalling in the ventral foregut Loss of Nkx2.1 expression in the ventral foregut domain</td>
<td>[47]</td>
</tr>
<tr>
<td>Barx1</td>
<td></td>
<td>Antagonist of canonical Wnt signaling</td>
<td>Barx1&lt;sup&gt;+/+&lt;/sup&gt; mice</td>
<td>Failure of foregut separation</td>
<td>[49]</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Loss of p63 and expansion of Nkx2.1 in the dorsal foregut domain</td>
<td></td>
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<tr>
<td><strong>RA</strong></td>
<td>Rar</td>
<td>Retinoic acid receptor</td>
<td>Rara1&lt;sup&gt;+/+&lt;/sup&gt;β&lt;sup&gt;+/+&lt;/sup&gt; mice</td>
<td>Failure of foregut separation. Undivided foregut is lined with ciliated epithelium</td>
<td>[51]</td>
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Table 2: Clinical trials investigating modulators of SHH and WNT signaling.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Agent</th>
<th>Study type</th>
<th>Primary outcome measure</th>
<th>Status</th>
<th>ClinicalTrials.gov identifier</th>
<th>Reference</th>
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<tr>
<td>SHH</td>
<td>BMS-833923 (Hedgehog inhibitor) with Cisplatin and Capecitabine</td>
<td>Phase 1</td>
<td>Determine maximum therapeutic dose, dose limiting toxicity and safety of BMS-833923 administered in combination with Cisplatin and Capecitabine</td>
<td>Completed</td>
<td>NCT00909402</td>
<td>[124]</td>
</tr>
<tr>
<td></td>
<td>PF-04449913 (Hedgehog inhibitor)</td>
<td>Phase 1</td>
<td>Determine first cycle dose limiting toxicity of PF-04449913 in patients with solid tumors</td>
<td>Completed</td>
<td>NCT01286467</td>
<td>[121]</td>
</tr>
<tr>
<td></td>
<td>LEQ506  (Hedgehog inhibitor)</td>
<td>Phase 1</td>
<td>Determine maximum tolerated dose and characterize dose limiting toxicity of LEQ506 in patients with solid tumors</td>
<td>Ongoing</td>
<td>NCT01106508</td>
<td>[122]</td>
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<td>TAK-441 (Hedgehog inhibitor)</td>
<td>Phase 1</td>
<td>Determine safety profile and pharmacokinetics of TAK-441 in patients with solid tumors with good performance status</td>
<td>Completed</td>
<td>NCT01204073</td>
<td>[120]</td>
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<td>Vismodegib (Hedgehog inhibitor)</td>
<td>Phase 1</td>
<td>Evaluate pharmacokinetics of Vismodegib in patients with solid tumors with good performance status</td>
<td>Recruiting</td>
<td>NCT01546519</td>
<td>[119]</td>
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<td>Vismodegib</td>
<td>Phase 2</td>
<td>Effect of Vismodegib on median progression-free survival of patients with advanced or recurrent stomach cancer</td>
<td>Ongoing</td>
<td>NCT00982592</td>
<td>[123]</td>
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<td>WNT</td>
<td>OMP-54F28 (Wnt pathway inhibitor)</td>
<td>Phase 1</td>
<td>Determine safety profile of OMP-54F28 in patients with solid tumors with good performance status</td>
<td>Recruiting</td>
<td>NCT01608867</td>
<td>[129]</td>
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<td>PRI-724 (Wnt pathway inhibitor)</td>
<td>Phase 1</td>
<td>Determine maximum therapeutic dose of PRI-724 in patients with solid tumors</td>
<td>Recruiting</td>
<td>NCT01302405</td>
<td>[128]</td>
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<td>Celecoxib with Irinotecan, Cisplatin and Radiation therapy versus Irinotecan, Cisplatin and Radiation therapy</td>
<td>Phase 2</td>
<td>Rate of cellular apoptosis and proliferation, rate of complete pathological remission in patients with resectable disease</td>
<td>Completed</td>
<td>NCT00520091</td>
<td>[127]</td>
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<td>Celecoxib with Paclitaxel and Carboplatin versus Paclitaxel and carboplatin</td>
<td>Phase 2</td>
<td>Pathological response at the time of surgical resection in patients with irresectable disease</td>
<td>Completed</td>
<td>NCT00066716</td>
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<td>Esomeprazole versus Esomeprazole and Aspirin (AspECT)</td>
<td>Phase 3</td>
<td>All causes of mortality or conversion rate from Barrett's metaplasia to adenocarcinoma</td>
<td>Ongoing</td>
<td>NCT00357682</td>
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<td>Vismodegib (Hedgehog inhibitor)</td>
<td>Phase 1</td>
<td>Evaluate pharmacokinetics of Vismodegib in patients with solid tumors with good performance status</td>
<td>Recruitng</td>
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