Advances in Biological Regulation xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Advances in Biological Regulation

journal homepage: www.elsevier.com/locate/jbior

Interaction of the Wnt/β-catenin and RAS-ERK pathways involving co-stabilization of both β-catenin and RAS plays important roles in the colorectal tumorigenesis

Sang-Kyu Lee[a,b], Jeong-Ha Hwang[a,b], Kang-Yell Choi[a,b,*]

[a] Translational Research Center for Protein Function Control, Yonsei University, Seoul, South Korea
[b] Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul, South Korea

ARTICLE INFO

Keywords:
Wnt/β-catenin signaling
RAS/ERK signaling
Colorectal cancer
Crosstalk
APC
RAS degradation

ABSTRACT

Cancer development is usually driven by multiple genetic and molecular alterations rather than by a single defect. In the human colorectal cancer (CRC), series of mutations of genes are involved in the different stages of tumorigenesis. For example, *adenomatous polyposis coli* (*APC*) and *KRAS* mutations have been known to play roles in the initiation and progression of the tumorigenesis, respectively. However, many studies indicate that mutations of these two genes, which play roles in the Wnt/β-catenin and RAS-extra-cellular signal regulated kinase (ERK) pathways, respectively, cooperatively interact in the tumorigenesis in several different cancer types including CRC. Both *Apc* and *Kras* mutations critically increase number and growth rate of tumors although single mutation of these genes does not significantly enhance the small intestinal tumorigenesis of mice. Both *APC* and *KRAS* mutations even result in the liver metastasis with inductions of the cancer stem cells (CSCs) markers in a mice xenograft model. In this review, we are going to describe the history for interaction between the Wnt/β-catenin and RAS/ERK pathways especially related with CRC, and provide the mechanical basis for the cross-talk between the two pathways. The highlight of the crosstalk involving the stability regulation of RAS protein via the Wnt/β-catenin signaling which is directly related with the cellular proliferation and transformation will be discussed. Activation status of GSK3β, a key enzyme involving both β-catenin and RAS degradations, is regulated by the status of the Wnt/β-catenin signaling dependent upon extracellular stimuli or intracellular abnormalities of the signaling components. The levels of both β-catenin and RAS proteins are co-regulated by the Wnt/β-catenin signaling, and these proteins are overexpressed with a positive correlation in the tumor tissues of CRC patients. These results indicate that the elevation of both β-catenin and RAS proteins is pathologically significant in CRC. In this review, we also will discuss further involvement of the increments of both β-catenin and RAS especially mutant KRAS in the activation of CSCs and metastasis. Overall, the increments of β-catenin and RAS especially mutant KRAS by *APC* loss play important roles in the cooperative tumorigenesis of CRC.

1. The Wnt/β-catenin pathway and cancer

The Wnt/β-catenin signaling pathway, which is often called as the canonical Wnt pathway, is activated by the combinatory

* Corresponding author. Translational Research Center for Protein Function Control, Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul, South Korea.
E-mail address: kychoi@yonsei.ac.kr (K.-Y. Choi).

https://doi.org/10.1016/j.jbior.2018.01.001
Received 6 January 2018; Received in revised form 8 January 2018; Accepted 8 January 2018

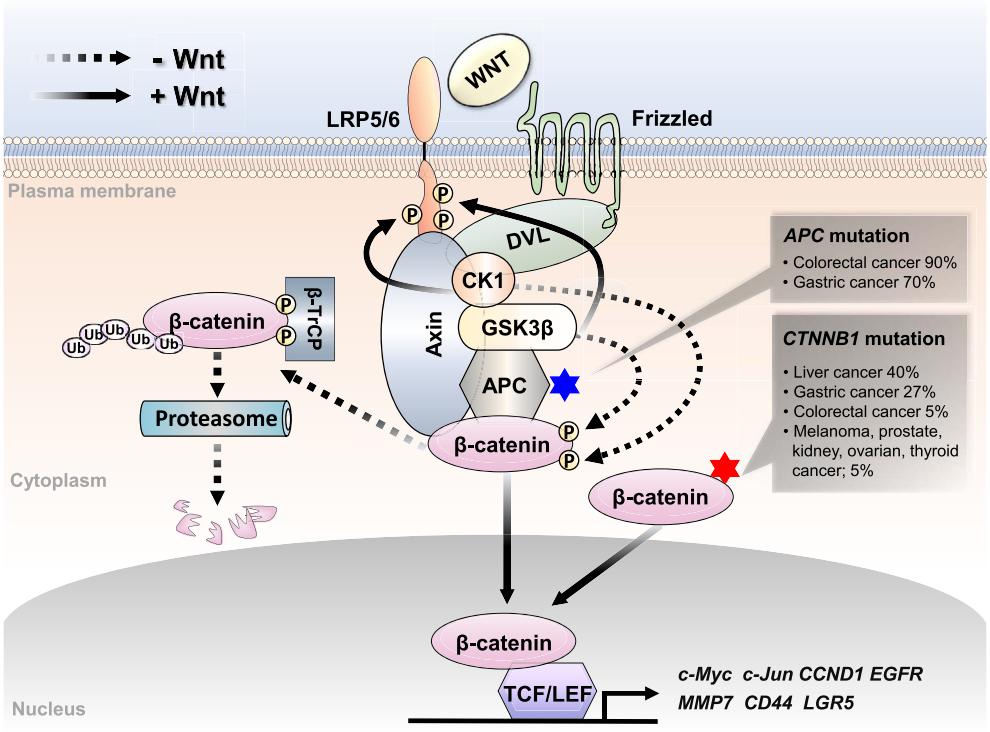

Fig. 1. Overview of the canonical Wnt/β-catenin pathway and gene mutations frequently found in human cancers.
In the absence of Wnt stimuli, the cytosolic β-catenin forms a complex with the destruction complex composed of the scaffolding protein Axin, APC, CK1, and GSK3β. The β-catenin is phosphorylated by CK1 and GSK3β, and subsequently recruits the E3 linker β-TrCP, resulting in the polyubiquitinylation-dependent proteasomal degradation of β-catenin (dotted lines). Upon binding of Wnt ligands to the receptor complex composed of Frizzled and its co-receptor LRP5/6, a signaling cascade is initiated activation of the canonical Wnt/β-catenin signaling. The LRP5/6 co-receptors are phosphorylated by the CK1 and GSK3β, which recruit the scaffold protein DVL, resulting in recruitment of intact axin complex (solid lines). The cytosolic β-catenin is accumulated by stabilization due to absence of its phosphorylation, followed by its translocation into nucleus to form an active transcription factor complex with TCF, leading to transcriptional activation of a large set of target genes including *c-Myc*, *c-Jun*, *CCND1*, *EGFR*, *MMP7*, *CD44*, and *LGR5* (solid lines). The mutations of the Wnt/β-catenin pathway component genes such as *APC or CTNNB1* frequently occur during tumorigenesis. The loss-of function *APC* mutations (blue asterisk) occur in several human cancers such as CRC as 90% and gastric cancer, especially the intestinal type as 70%. The activating β-catenin mutations (red asterisk) are observed 30–40% of HCC, 27% of intestinal type of gastric cancer, and around 5% of CRC exclusively with the *APC* mutations. The black arrows with solid line indicate the events which occur in the presence of Wnt stimuli. The black arrows with dotted line indicate the events which occur in the absence of Wnt stimuli. The red and blue asterisks indicate the events which occur in the gain and loss of function mutations, respectively. Axin, axis inhibition protein; APC, adenomatous polyposis coli; CK1, casein kinase 1; GSK3β, glycogen synthase kinase 3β; LRP5/6, low-density lipoprotein receptor-related protein family; DVL, dishevelled; TCF, T cell factor. *P*, phosphorylation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

binding of the multiple Wnt ligands to the Frizzled (FZD) receptors at the cell surface, and these membranous signaling events are complicated and poorly illustrated. In the resting status without significant Wnt stimuli, the cytosolic β-catenin subjects to the degradation via formation of the "β-catenin destruction complex" composed of the scaffolding protein Axin, tumor suppressor adenomatous polyposis coli (APC), casein kinase 1 (CK1), and glycogen synthase kinase 3 beta (GSK3β) (MacDonald et al., 2009). The priming phosphorylation of β-catenin by CK1 and subsequent phosphorylations by GSK3β allow the recruitment of the E3 ligase linker β-TrCP, subsequently resulting in the degradation of β-catenin via the polyubiquitinylation-mediated proteasomal degradation (Amit et al., 2002; Liu et al., 2002) (Fig. 1). In the activation status, increased extracellular Wnt ligands bind to the FZD receptors together with the low density lipoprotein receptor-related protein (LRP) family receptors, LRP5/6, and this initiates activation of the Wnt/β-catenin signaling. The LRP5/6 co-receptors are phosphorylated by the CK1 and GSK3β kinases, which recruit the scaffold protein Dishevelled (Dvl), resulting in further recruitment of intact axin complex (MacDonald et al., 2009; Tamai et al., 2000) (Fig. 1). This results in dissociation of the destruction complex followed by cytosolic accumulation of β-catenin via its stabilization due to absence of its phosphorylations. The β-catenin subsequently translocates into nucleus, forms the complex with the T cell factor (TCF) or lymphoma enhancer factor (LEF), and activates target genes. Currently, around 100 genes have been known as the target genes for the β-catenin signaling, and many of the genes such as *c-Myc* (He et al., 1998), *c-Jun* (Mann et al., 1999), *CCND1* (Tetsu and McCormick, 1999), *MMP7* (Brabletz et al., 1999), *VEGF* (Zhang et al., 2001), and *EGFR* (Guturi et al., 2012; Tan et al., 2005) are involved in the activation of proliferation and transformation of cells and cancers including CRC (Fig. 1).

Aberrant Wnt/β-catenin signaling activation is frequently observed in CRC, hepatocellular carcinoma (HCC), gastric cancer, lung cancer, melanoma, and breast cancer *etc*. (Clevers and Nusse, 2012; MacDonald et al., 2009; Polakis, 2000; Zhan et al., 2017). The mutations of the Wnt/β-catenin pathway component genes such as *APC* or *CTNNB1* (encoding β-catenin) frequently occur during

tumorigenesis (Fig. 1). For example, loss of function mutations of *APC* occur in several different types of human cancers such as CRC as 90% and gastric cancer, especially intestinal type as 70%, respectively (Lee et al., 2002; Yuasa, 2003). The activating β-catenin mutations, which are responsible for impaired its proteasomal degradation due to mutations of the phosphorylation sites on the amino terminal region of the protein required for recognition of the E3 linker β-TrCP, are observed 30–40% of human HCC, and 27% of intestinal type of gastric cancer (de La Coste et al., 1998; Fodde, 2002; Miyoshi et al., 1998; Polakis, 1999). The pathophysiological importance of Wnt/β-catenin signaling in cancer development is well-studied in CRC. Loss of tumor suppressor *APC*, resulting in activation of Wnt/β-catenin signaling via β-catenin stabilization, is the major driver in colorectal tumorigenesis (Kinzler and Vogelstein, 1996; Zhan et al., 2017). The *CTNNB1* mutations are rare in CRC and its mutations are found only 5% of CRC patient tissues without overlapping *APC* mutations (Morin, 1999). In addition, the negative regulators of the Wnt/β-catenin pathway functioning at the cell surface such as secreted frizzled-related proteins (sFRPs), Wnt inhibitory factor (WIF), and Dickkopf-related protein (DKK) are often silenced in tumor cells through promoter methylation or histone modification (Ying and Tao, 2009).

Aberrant activation of Wnt/β-catenin signaling pathway is associated with activation and maintenance of cancer stem cells (CSCs) with self-renewal capacity and plasticity that are responsible for fueling tumor growth (de Sousa e Melo and Vermeulen, 2016; Zhan et al., 2017). Such as, *APC* loss 수정 is also known to be involved in the maintenance and activation of stemness of CSCs, and several CSCs markers such as CD44, CD133, and leucine-rich-repeat containing G-protein-coupled receptor 5 (Lgr5) are target genes of Wnt/β-catenin signaling (Barker et al., 2007, 2009; Brabletz et al., 2009; Lewis et al., 2010; Wielenga et al., 1999) (Fig. 1). The LGR5 receptor, a potential marker of intestinal stem cells, binds to R-spondin with high affinity that activates the FZD-LRP5/6 complex together with Wnt ligand, resulting in the further activation of CSCs by Wnt/β-catenin signaling (Carmon et al., 2011; de Lau et al., 2011). Elevated levels of the Wnt/β-catenin signaling target genes such as, LGR5 and CD44 are observed in primary and metastatic CRCs, and many other malignancies related with activation of CSCs (Dalerba et al., 2007; Kemper et al., 2012; Yan et al., 2015). In addition, the alterations of the R-spondin/Lgr5/RNF43 axis are implicated as driver of Wnt-dependent tumor growth and maintenance of cancer stemness (Giannakis et al., 2014; Seshagiri et al., 2012; Zhan et al., 2017).

2. RAS signaling pathway and cancer

The RAS pathway activation is initiated by upstream signaling involving binding of growth factors to the receptor tyrosine kinase (RTKs) such as binding of epidermal growth factor (EGF) to the EGF receptor (EGFR) resulting in the conformation change of EGFR accompanying its dimerization (Marshall, 1995; Porter and Vaillancourt, 1998; Schlessinger, 1993). The RTKs activated by dimerization induce autophosphorylations at the multiple tyrosine residues, and that provide docking sites for several different proteins with src homology 2 (SH2) domain such as p110 subunit of phosphatidylinositide 3-kinases (PI3K), Shc, SHP-2, and growth factor receptor bound protein 2 (GRB2) *etc.* (Porter and Vaillancourt, 1998; Reuter et al., 2000). Autophosphorylated receptor binds to the SH2 domain of Grb2, which is bound to Sos via its SH3 domain, followed by activation of RAS by exchanging GDP to GTP guanine nucleotide (Porter and Vaillancourt, 1998; Reuter et al., 2000; Schlessinger, 1993) (Fig. 2). The activated GTP bound RAS further transmits its signal to the downstream RAF-MEK-ERK and PI3K-Akt signaling cascades (Daum et al., 1994; Van Aelst et al., 1994). The RAF-MEK-ERK signaling, also known as the mitogen-activated protein kinase (MAPK) cascade, is activated by series of multiple phosphorylation events of the kinases (Downward, 2003). The PI3K-Akt pathway is also activated by active RAS, and induces proliferation and other cellular physiologies (Carpenter and Cantley, 1996; Rodriguez-Viciana et al., 1994). The ERK proteins activated by their phosphorylations are translocated into nucleus for activation of transcription factors such Ets, Elk-1, and Myc which activate many target genes involving proliferation, survival, movement and differentiation of cells (Dhillon et al., 2007) (Fig. 2).

The RAS signaling pathway is aberrantly activated by both mutations and overexpression of the upstream RTKs including the EGFR and ERBB2. These abnormalities have been observed in breast, lung, ovarian, gastric, pancreatic, and colon cancer (Downward, 2003; Fitzgerald et al., 2015; Hu and Li, 2010; Janku et al., 2010; Mendelsohn and Baselga, 2000). Aberrant activation of RAS protein itself, frequently induced by mutations at the specific sites most frequently at the amino acid numbers 12, 13 and 61 *etc.*, locking RAS as active GTP-bound forms, is major abnormality found human cancers. Their mutations are found in most of human cancers such as 40–50% and 90% of *KRAS* mutations in the CRC and pancreatic cancers, respectively (Downward, 2003; Gysin et al., 2011; Kinzler and Vogelstein, 1996; Reuter et al., 2000) (Fig. 2). The RAS mutations result in the hyper-activation of the Raf-MEK-ERK signaling pathway as well as the PI3K-Akt pathway involving transformation of cells and tumorigenesis (Downward, 2003) (Fig. 2). In addition, RAS signaling can be activated by loss of the GTPase activating proteins (GAPs) or mutations of *BRAF*, a downstream RAS effector in many different cancer types (Downward, 2003).

3. The Wnt/β-catenin and RAS-ERK pathways synergistically interact in the tumorigenesis

Although the Wnt/β-catenin and RAS-ERK pathways are known to play important roles in the transformation of cells and caner, mutations of the genes in each of these two pathways often do not result in significant cancer phenotypes. However, multiple mutations of both pathways genes critically enhance many different stages of the tumorigenesis including initiation, progression, and metastasis (Fearon and Vogelstein, 1990; Phelps et al., 2009) (Fig. 3). For example, HCC develops at the 100% incidence in mice with mutations of both *CTNNB1* and *Hras* although *Hras* mutation alone does not result in tumor phenotypes with the large cell dysplasia in the hepatocytes (Harada et al., 2004). The colon cancer carcinomas are synergistically induced by both loss of function *Apc* mutations and activated *Kras* mutations (D'Abaco et al., 1996). In addition, the plasticity and self-renewal capacity of tumor as well as tumor multiplicity and malignant behavior are critically increased in the compound mutant mice with both oncogenic *Kras* and loss-of-function *Apc* mutations (Janssen et al., 2006). We also observed critical enhancement of both initiation and growth of small

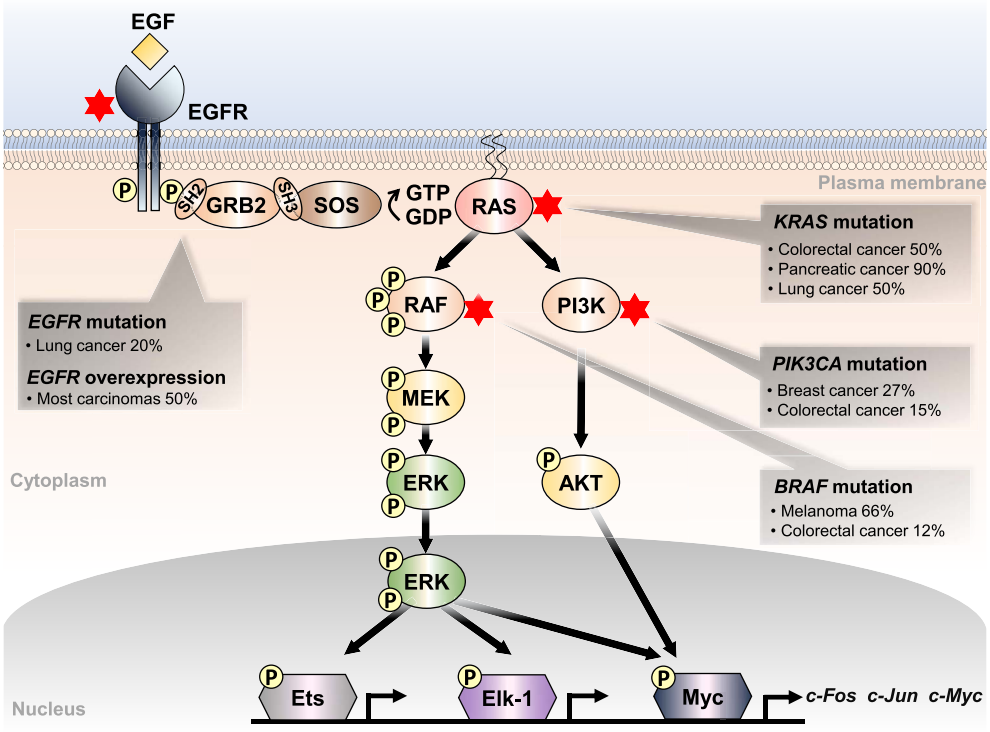

Fig. 2. Overview of the RAS/MAPK singling pathway and gene mutations frequently found in human cancers.
The RAS/MAPK signaling pathway activation is initiated by binding of growth factor such as EGF to the receptor tyrosine kinase (RTK) such as EGFR. The activation of RTKs by its dimerization and autophosphorylations serves as docking sites for SH2 domain of GRB2. GRB2 binds to SOS via its SH3 domains to activate RAS by exchanging GDP to GTP guanine nucleotide. The activated GTP bound RAS activates its signal to the downstream both RAF-MEK-ERK and PI3K-Akt signaling cascades. The active RAS recruits and activates RAF at the plasma membrane, which then phosphorylates MEK. Activated MEK subsequently phosphorylates ERK, which translocates to the nucleus where it phosphorylates and activates various transcription factors including Ets, Elk-1, and Myc, which activate target genes including *c-Fos*, *c-Jun*, and *c-Myc* etc. The RAS signaling pathway is aberrantly activated by both mutations and overexpressions of components of EGFR-RAS-MAPK signaling pathway in various cancers. The *KRAS* mutations occur in most human cancers such as CRC as 50%, pancreatic cancers as 90%, and lung cancer as 50%. The overexpression of EGFR is frequently observed in most carcinomas (approximately 50%) and the *EGFR* mutations occur in lung cancer as 23% and gastric cancer as 10%. Furthermore, *RAF* mutations are frequently observed in melanomas (66%), thyroid (40%), CRC (12%), and ovarian (10%) cancer. The red asterisks indicate the gain of function mutations. EGF, epidermal growth factor; EGFR, EGF receptor; GRB2, growth factor receptor-bound protein-2; SOS, son of sevenless; MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide 3-kinase; *P*, phosphorylation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

intestinal tumors by strong activation of the Wnt/β-catenin signaling induced by both *Apc* and *Kras* mutations (Jeong et al., 2012; Moon et al., 2014) (Fig. 3). Although oncogenic *Kras* mutations alone do not activate CSCs, additional *Apc* mutations significantly induce several CSCs makers such as CD44, CD133 and CD166 in the $Apc^{Min/+}$ and $Kras^{LA2}$ compound mouse model (Moon et al., 2014). Both *Apc* and *Kras* mutations even result in the liver metastasis of a xenograft mouse model using transforming CRC cells with both *APC* and *KRAS* mutations (Moon et al., 2014). All of these indicate that *APC* and *KRAS* mutations synergistically interact in tumorigenesis at the many different stages.

4. The mechanism for cross-talk between the Wnt/β-catenin and RAS-ERK pathways; RAS stabilization via the Wnt/β-catenin pathway

The interaction between Wnt/β-catenin and RAS/ERK pathways has been evidenced. The RAF-1-MEK-ERK pathway is immediately activated by recombinant Wnt3a treatment in NIH3T3 and L cells, indicated direct interaction of the Wnt/β-catenin and RAF-1-MEK-ERK pathways (Yun et al., 2005). The PI3K and ERK pathways are also activated by Wnt3a, and that is related with the cellular proliferation (Kim and Choi, 2007; Kim et al., 2007). Our further series of studies have been proved regulation of the RAF-1-MEK-ERK signaling cascade by the Wnt/β-catenin signaling (Jeon et al., 2007a, 2007b; Park et al., 2006). In addition, GSK3β, key component of Wnt/β-catenin pathway, is regulated by RAS-MAPK and PI3K-Akt signaling cascades, and the cross-talk between GSK3β and RAS-MAPK or PI3K/AKT/mTOR signaling pathways is implicated in the pathogenesis of HCC and pancreatic cancer (Cervello et al., 2017; Fitzgerald et al., 2015; Hermida et al., 2017).

A key mechanism for the cross-talk between the Wnt/β-catenin and RAF-1-MEK-ERK pathways is the regulation of RAS protein stability via the status of Wnt/β-catenin signaling (Fig. 4); HRAS is subjected to the degradation via the Wnt/β-catenin signaling through the β-TrCP E3 linker-mediated polyubiquitinylation-dependent proteasomal degradation (Kim et al., 2009). The HRAS

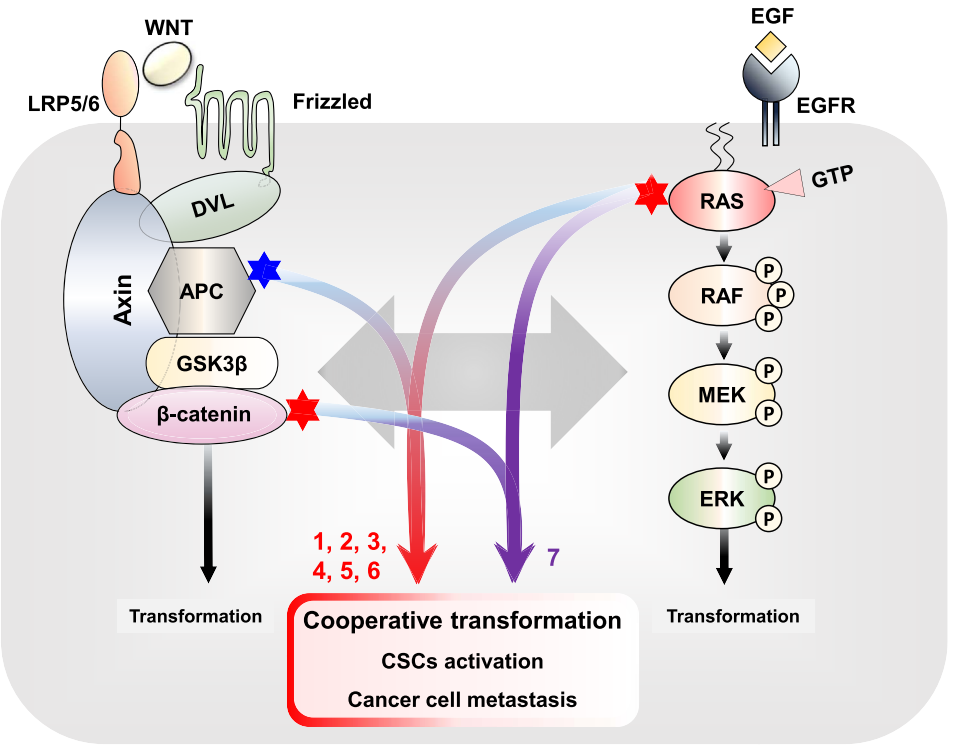

Fig. 3. Schematic overview of interaction between Wnt/β-catenin and RAS/ERK pathways in cancer.
The single mutation of components of Wnt/β-catenin and RAS-ERK pathways often does not induce significant transformation associated with cancer. However, the multiple mutations of both pathway genes critically enhance cooperative the tumorigenesis including initiation and progression (1, Fearon and Vogelstein, 1990; 2, Phelps et al., 2009). The small intestinal tumors are synergistically induced by both *Apc* and *Kras* mutations in mice model (3, D'Abaco et al., 1996; 4, Janssen et al., 2006). Although oncogenic *KRAS* mutations alone do not activate CSCs in the mice model, both *APC* and *KRAS* mutations significantly induce small intestinal tumors accompanying the activation of CSCs via induction of CSCs markers, and even result in the liver metastasis of a xenograft mouse model (5, Jeong et al., 2012; 6, Moon et al., 2014). In addition, *CTNNB1* and *HRAS* mutations are cooperatively induced hepatocellular carcinoma (7, Harada et al., 2004). The merged arrows indicates synergistic signaling pathways derived from the multiple mutations of components of both Wnt/β-catenin and RAS-ERK pathways. The red and blue asterisks indicate the gain- and loss-of-function gene mutations, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

stability regulation via the Wnt/β-catenin signaling is modulated by Axin or APC overexpression or by recombinant Wnt3a treatment, and that is directly related with the transformation of CRC cells (Kim et al., 2009). By further characterization, GSK3β is identified as a kinase inducing phosphorylations of HRAS at the threonine (Thr)-144 and Thr-148 residues, and that subsequently recruits the β-TrCP E3 linker for the proteasomal degradation (Jeong et al., 2012; Kim et al., 2009) (Fig. 4, upper panel). In the Wnt stimuli or genetic alterations such as *APC* loss, GSK3β becomes inactive status, resulting in RAS stabilization due to absence of the phosphorylations and subsequent polyubiquitinylation-dependent proteasomal degradation (Fig. 4, lower panel). The Thr-144 and Thr-148 residues are conserved in all H-, N-, and K-RAS, and all of these RAS isotypes are subjected to the stability regulation by the Wnt/β-catenin signaling (Jeong et al., 2012). Due to usage of common kinase GSK3β in their phosphorylations, β-catenin and RAS stabilities are co-regulated by the status of Wnt/β-catenin signaling. Therefore, both β-catenin and RAS levels are highly increased by *APC* loss (Jeong et al., 2012).

5. Stabilizations of both β-catenin and RAS especially mutant KRAS by *APC* loss are a mechanical base for the synergistic transformation of CRC cells and CSCs activation by both *APC* and *KRAS* mutations

The stability regulations of both β-catenin and RAS are positively correlated with the Wnt/β-catenin signaling involving the proliferation of CRC cells, and the synergistic effect on cellular transformation by both *APC* and *KRAS* mutations is attributed by the stabilization of oncogenic mutant KRAS as well as β-catenin (Fig. 5). By the co-stabilizations of oncogenic KRAS and β-catenin by both *Apc* and *Kras* mutations are critically enhanced both initiation and progression of CRC in the xenograft and transgenic mice models (Jeong et al., 2012; Moon et al., 2014). Each *Apc* or *Kras* mutations dose not reveal or mild cancer phenotypes with the weak CSCs activation (Fig. 5). However, the strong CSCs activation and even metastasis are observed in mice harboring both *Apc* and *Kras* mutations via stabilizations of both β-catenin and oncogenic KRAS inducing activations of ERK (Moon et al., 2014) (Fig. 5). The pathological significance of the co-stabilization of β-catenin and RAS in tumorigenesis is indicated by high levels of β-catenin and RAS in CRC patient tissues (Jeong et al., 2012; Moon et al., 2014), where *APC* mutations occur as high as 90%. The RAS stabilization,

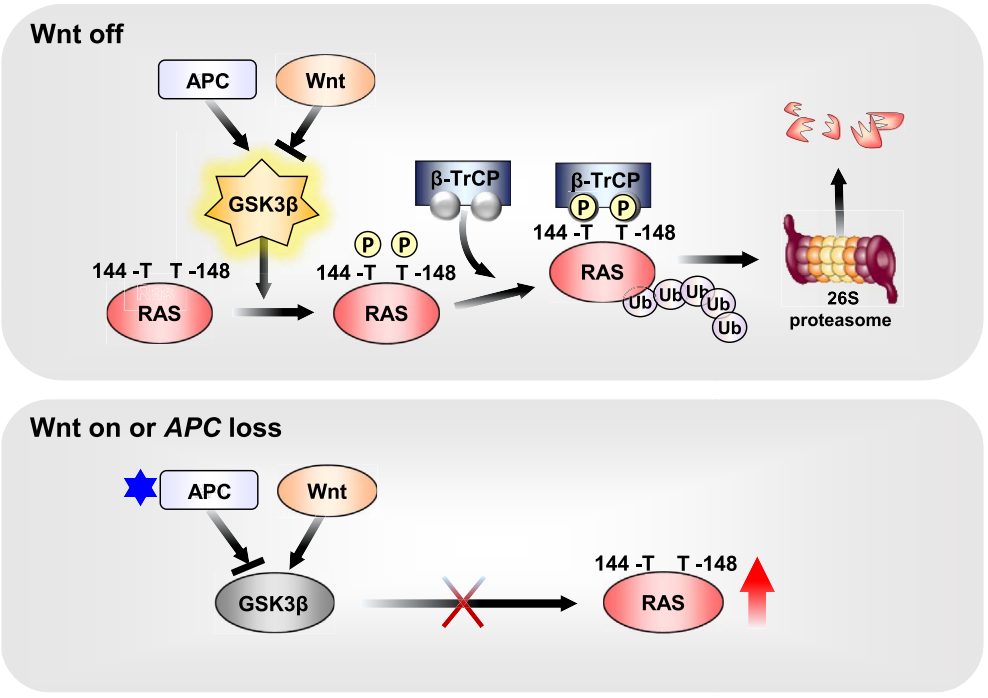

Fig. 4. Schematic mechanisms of RAS stability regulation via Wnt/β-catenin signaling.
In Wnt off status, the activated GSK3β phosphorylates RAS at Thr-144 and Thr-148, and subsequently recruits β-TrCP E3 linker for the polyubiquitinylation-dependent proteasomal degradation (upper panel). In Wnt on status or *APC* loss, GSK3β becomes inactive status, consequently resulting in the stabilization of RAS due to absence of its phosphorylation and polyubiquitinylation-dependent proteasomal degradation (lower panel). The blue asterisk indicates the loss-of-function mutation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

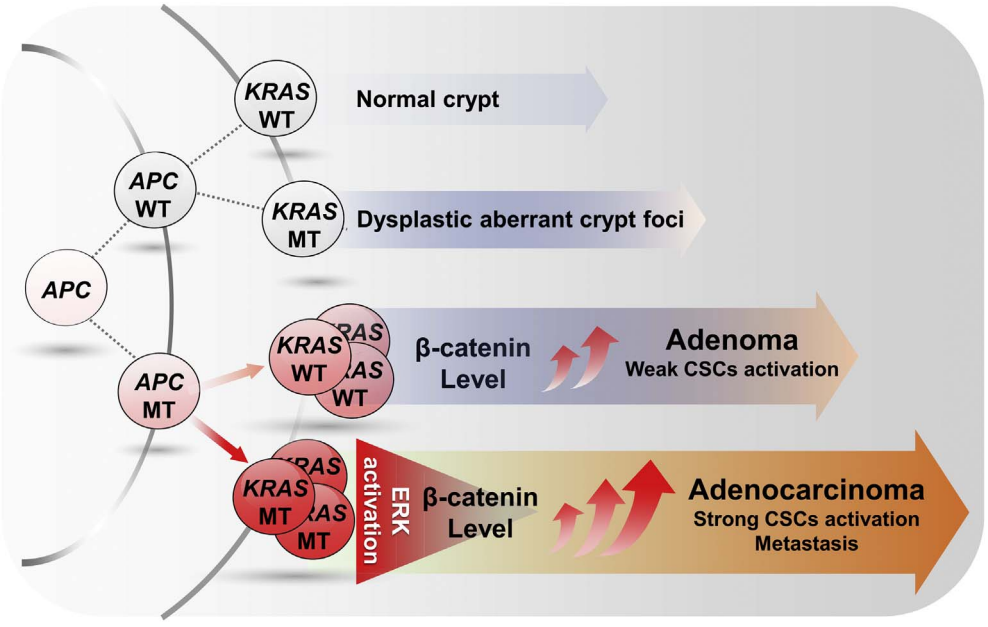

Fig. 5. Schematic model for multi-step process of synergistic transformation and tumorigenesis attributed by co-stabilization of β-catenin and RAS by *APC* loss.
Although the loss of function *APC* mutations are important for initiation of neoplasia process, the single mutation of *APC* or *KRAS* does not result in significant cancer phenotypes. *KRAS* mutations may contribute to dysplastic aberrant crypt foci formation, and *APC* mutation only induces adenoma along with weak activation of CSCs via stabilization and transactivation of β-catenin. The phenotypes by *APC* mutations alone may be caused by stabilization of wild-type KRAS as well as that of β-catenin. Both *APC* and *KRAS* mutations cooperatively induce adenocarcinoma accompanying with strong CSCs activation and even metastasis via stabilizations of both β-catenin and oncogenic mutant KRAS, followed by hyper-activation of ERK and further β-catenin transactivation.

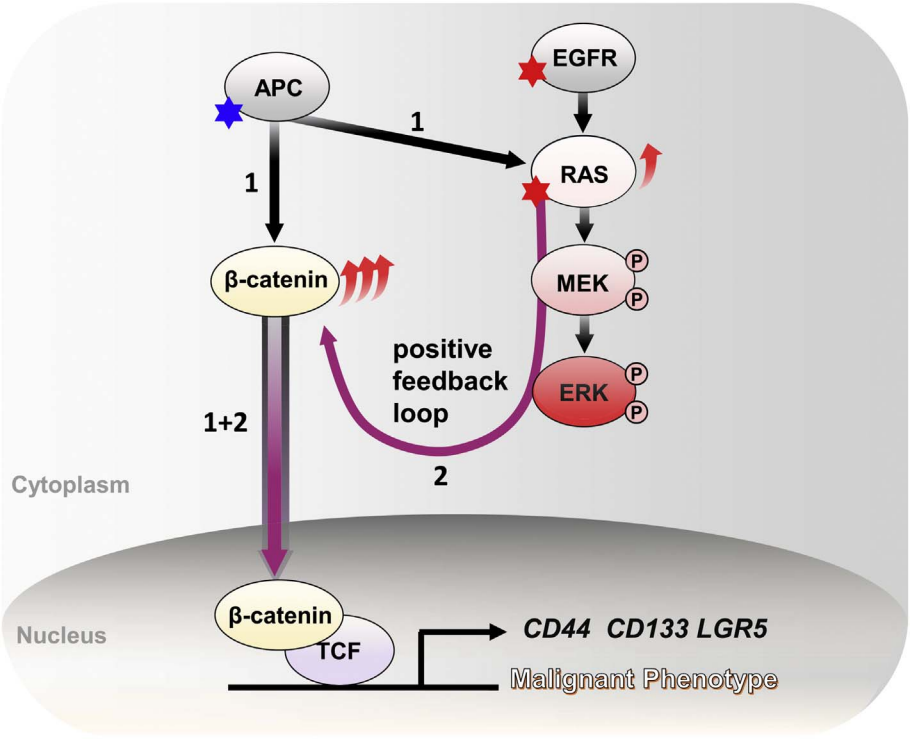

Fig. 6. Schematic mechanism for the positive cooperation between Wnt/β-catenin and RAS/ERK signaling pathways on CSCs activation and malignant transformation. *APC* mutations initially stabilize both β-catenin and RAS, resulting in activations of both Wnt/β-catenin and RAS/ERK signaling pathways (1, Jeong et al., 2012). Stabilization of mutant KRAS by *APC* loss further activates β-catenin through the positive feedback loop via MEK-ERK pathway (2, Moon et al., 2014). The increased β-catenin translocates into nucleus, and induces target genes including CSCs markers such as the *CD44*, *CD133*, and *LGR5* and *EGFR* for the phenotypes of the malignant transformation including metastasis (1 + 2, Moon et al., 2014). The red and blue asterisks indicate gain- and loss-of-function mutations, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

especially mutant RAS, which also occurs as 40–50% of CRC, plays critical roles in the colorectal pathogenesis involving both *APC* and *KRAS* mutations (Jeong et al., 2012; Moon et al., 2014).

The EGFR, which is direct transcriptional target of β-catenin, is frequently mutated and overexpressed in most carcinomas, and these with the aberrant activation of Wnt/β-catenin pathway are responsible for synergistic tumorigenesis of the different types of human cancer including CRC (Gross et al., 1991; Hu and Li, 2010; Radinsky et al., 1995). The generation of secondary mutations after treatments of the anti-cancer therapies especially those targeting EGFR is a major huddle to be overcome (Benavente et al., 2009; Wheeler et al., 2008; Yonesaka et al., 2011). Moreover, EGFR targeting drugs reveal limitations in their usage due to their poor efficacy or insensitivity to the patients harboring *KRAS* mutations (Amado et al., 2008; Fitzgerald et al., 2015; Karapetis et al., 2008; Lievre et al., 2008). The significance of *KRAS* mutations especially with the preceding *APC* mutations is highlighted by activation of CSCs, and that is acquired by initial activation of β-catenin by *APC* loss (1), followed by its secondary strong activation by the stabilized oncogenic KRAS via a positive feedback loop through MEK-ERK pathway (2) (Moon et al., 2014, Fig. 6). The increased β-catenin by this dual activation mechanism translocates into nucleus for the activation of the target genes such as *CD44*, *CD133*, *LGR5* CSCs marker genes and *EGFR* (Fig. 6). Together, stabilizations of both β-catenin and mutant KRAS as well as the transcriptional induction of EGFR especially its mutant form by *APC* mutations cooperatively enhance Wnt/β-catenin signaling, which in turn synergistic activation of CSCs and metastasis of CRCs.

6. Concluding remarks

In this review, we summarized the current understanding of cross-talk between Wnt/β-catenin and RAS/ERK pathways related with the synergism in the pathogenesis of CRC by *APC* and *KRAS* mutations, which are most frequently found in human CRC. The highlight of the cross-talk is the stabilizations of both β-catenin and RAS proteins especially oncogenic mutant KRAS, which are a mechanical basis of the synergism by both *APC* and *KRAS* mutations in colorectal tumorigenesis including initiation, progression, and metastasis involving activation of CSCs. Overall, targeting both pathways via lowering both β-catenin and RAS proteins can be a potential therapy for the treatment of CRC patients with aberrantly activated Wnt/β-catenin and EGFR/RAS/ERK pathways attributed to the increments of β-catenin and RAS as well as their activation by pathologically important *APC* and *KRAS* mutations.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant (2016R1A5A1004694, 2015R1A2A1A05001873) funded by the Korean Government (MSIP).

Author declaration

The authors declare no competing financial interests.

Conflicts of interest

The authors declare no competing financial interests.

References

Amado, R.G., Wolf, M., Peeters, M., Van Cutsem, E., Siena, S., Freeman, D.J., Juan, T., Sikorski, R., Suggs, S., Radinsky, R., Patterson, S.D., Chang, D.D., 2008. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J. Clin. Oncol. 26, 1626–1634.

Amit, S., Hatzubai, A., Birman, Y., Andersen, J.S., Ben-Shushan, E., Mann, M., Ben-Neriah, Y., Alkalay, I., 2002. Axin-mediated CKI phosphorylation of β-catenin at Ser 45: a molecular switch for the Wnt pathway. Gene Dev. 16, 1066–1076.

Barker, N., Ridgway, R.A., van Es, J.H., van de Wetering, M., Begthel, H., van den Born, M., Danenberg, E., Clarke, A.R., Sansom, O.J., Clevers, H., 2009. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 457, 608–611.

Barker, N., Van Es, J.H., Kuipers, J., Kujala, P., Van Den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., 2007. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449, 1003–1007.

Benavente, S., Huang, S., Armstrong, E.A., Chi, A., Hsu, K.T., Wheeler, D.L., Harari, P.M., 2009. Establishment and characterization of a model of acquired resistance to epidermal growth factor receptor targeting agents in human cancer cells. Clin. Canc. Res. 15, 1585–1592.

Brabletz, S., Schmalhofer, O., Brabletz, T., 2009. Gastrointestinal stem cells in development and cancer. J. Pathol. 217, 307–317.

Brabletz, T., Jung, A., Dag, S., Hlubek, F., Kirchner, T., 1999. beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. Am. J. Pathol. 155, 1033–1038.

Carmon, K.S., Gong, X., Lin, Q., Thomas, A., Liu, Q., 2011. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. Proc. Natl. Acad. Sci. U S A 108, 11452–11457.

Carpenter, C.L., Cantley, L.C., 1996. Phosphoinositide kinases. Curr. Opin. Cell Biol. 8, 153–158.

Cervello, M., Augello, G., Cusimano, A., Emma, M.R., Balasus, D., Azzolina, A., McCubrey, J.A., Montalto, G., 2017. Pivotal roles of glycogen synthase-3 in hepatocellular carcinoma. Adv. Biol. Regul. 65, 59–76.

Clevers, H., Nusse, R., 2012. Wnt/beta-catenin signaling and disease. Cell 149, 1192–1205.

D'Abaco, G.M., Whitehead, R.H., Burgess, A.W., 1996. Synergy between Apc min and an activated ras mutation is sufficient to induce colon carcinomas. Mol. Cell Biol. 16, 884–891.

Dalerba, P., Dylla, S.J., Park, I.K., Liu, R., Wang, X., Cho, R.W., Hoey, T., Gurney, A., Huang, E.H., Simeone, D.M., Shelton, A.A., Parmiani, G., Castelli, C., Clarke, M.F., 2007. Phenotypic characterization of human colorectal cancer stem cells. Proc. Natl. Acad. Sci. U S A 104, 10158–10163.

Daum, G., Eisenmann-Tappe, I., Fries, H.-W., Troppmair, J., Rapp, U.R., 1994. The ins and outs of Raf kinases. Trends Biochem. Sci. 19, 474–480.

de La Coste, A., Romagnolo, B., Billuart, P., Renard, C.A., Buendia, M.A., Soubrane, O., Fabre, M., Chelly, J., Beldjord, C., Kahn, A., Perret, C., 1998. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. Proc. Natl. Acad. Sci. U S A 95, 8847–8851.

de Lau, W., Barker, N., Low, T.Y., Koo, B.-K., Li, V.S., Teunissen, H., Kujala, P., Haegebarth, A., Peters, P.J., van de Wetering, M., 2011. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature 476, 293–297.

de Sousa e Melo, F., Vermeulen, L., 2016. Wnt signaling in cancer stem cell biology. Cancers 8, 60.

Dhillon, A.S., Hagan, S., Rath, O., Kolch, W., 2007. MAP kinase signalling pathways in cancer. Oncogene 26, 3279–3290.

Downward, J., 2003. Targeting RAS signalling pathways in cancer therapy. Nat. Rev. Canc. 3, 11–22.

Fearon, E.R., Vogelstein, B., 1990. A genetic model for colorectal tumorigenesis. Cell 61, 759–767.

Fitzgerald, T.L., Lertpiriyapong, K., Cocco, L., Martelli, A.M., Libra, M., Candido, S., Montalto, G., Cervello, M., Steelman, L., Abrams, S.L., 2015. Roles of EGFR and KRAS and their downstream signaling pathways in pancreatic cancer and pancreatic cancer stem cells. Adv. Biol. Regul. 59, 65–81.

Fodde, R., 2002. The APC gene in colorectal cancer. Eur. J. Canc. 38, 867–871.

Giannakis, M., Hodis, E., Jasmine Mu, X., Yamauchi, M., Rosenbluh, J., Cibulskis, K., Saksena, G., Lawrence, M.S., Qian, Z.R., Nishihara, R., Van Allen, E.M., Hahn, W.C., Gabriel, S.B., Lander, E.S., Getz, G., Ogino, S., Fuchs, C.S., Garraway, L.A., 2014. RNF43 is frequently mutated in colorectal and endometrial cancers. Nat. Genet. 46, 1264–1266.

Gross, M., Zorbas, M., Danels, Y., Garcia, R., Gallick, G., Olive, M., Brattain, M.G., Boman, B., Yeoman, L., 1991. Cellular growth response to epidermal growth factor in colon carcinoma cells with an amplified epidermal growth factor receptor derived from a familial adenomatous polyposis patient. Canc. Res. 51, 1452–1459.

Guturi, K.K.N., Mandal, T., Chatterjee, A., Sarkar, M., Bhattacharya, S., Chatterjee, U., Ghosh, M.K., 2012. Mechanism of β-catenin-mediated transcriptional regulation of epidermal growth factor receptor expression in glycogen synthase kinase 3 β-inactivated prostate cancer cells. J. Biol. Chem. 287, 18287–18296.

Gysin, S., Salt, M., Young, A., McCormick, F., 2011. Therapeutic strategies for targeting ras proteins. Gene Canc. 2, 359–372.

Harada, N., Oshima, H., Katoh, M., Tamai, Y., Oshima, M., Taketo, M.M., 2004. Hepatocarcinogenesis in mice with beta-catenin and Ha-ras gene mutations. Canc. Res. 64, 48–54.

He, T.C., Sparks, A.B., Rago, C., Hermeking, H., Zawel, L., da Costa, L.T., Morin, P.J., Vogelstein, B., Kinzler, K.W., 1998. Identification of c-MYC as a target of the APC pathway. Science 281, 1509–1512.

Hermida, M.A., Kumar, J.D., Leslie, N.R., 2017. GSK3 and its interactions with the PI3K/AKT/mTOR signalling network. Adv. Biol. Res. 65, 5–15.

Hu, T., Li, C., 2010. Convergence between Wnt-β-catenin and EGFR signaling in cancer. Mol. Canc. 9, 1.

Janku, F., Stewart, D.J., Kurzrock, R., 2010. Targeted therapy in non-small-cell lung cancer–is it becoming a reality? Nat. Rev. Clin. Oncol. 7, 401–414.

Janssen, K.P., Alberici, P., Fsihi, H., Gaspar, C., Breukel, C., Franken, P., Rosty, C., Abal, M., El Marjou, F., Smits, R., 2006. APC and oncogenic KRAS are synergistic in enhancing Wnt signaling in intestinal tumor formation and progression. Gastroenterology 131, 1096–1109.

Jeon, S.H., Jeong, W.J., Cho, J.Y., Lee, K.H., Choi, K.Y., 2007a. Akt is involved in the inhibition of cell proliferation by EGF. Exp. Mol. Med. 39, 491–498.

Jeon, S.H., Yoon, J.Y., Park, Y.N., Jeong, W.J., Kim, S., Jho, E.H., Surh, Y.J., Choi, K.Y., 2007b. Axin inhibits extracellular signal-regulated kinase pathway by Ras degradation via beta-catenin. J. Biol. Chem. 282, 14482–14492.

Jeong, W.J., Yoon, J., Park, J.C., Lee, S.H., Kaduwal, S., Kim, H., Yoon, J.B., Choi, K.Y., 2012. Ras stabilization through aberrant activation of Wnt/beta-catenin signaling promotes intestinal tumorigenesis. Sci. Signal. 5 ra30.

Karapetis, C.S., Khambata-Ford, S., Jonker, D.J., O'Callaghan, C.J., Tu, D., Tebbutt, N.C., Simes, R.J., Chalchal, H., Shapiro, J.D., Robitaille, S., Price, T.J., Shepherd, L., Au, H.J., Langer, C., Moore, M.J., Zalcberg, J.R., 2008. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N. Engl. J. Med. 359, 1757–1765.

Kemper, K., Prasetyanti, P.R., De Lau, W., Rodermond, H., Clevers, H., Medema, J.P., 2012. Monoclonal antibodies against Lgr5 identify human colorectal cancer stem cells. Stem Cell. 30, 2378–2386.
Kim, S.-E., Choi, K.-Y., 2007. EGF receptor is involved in WNT3a-mediated proliferation and motility of NIH3T3 cells via ERK pathway activation. Cell. Signal. 19, 1554–1564.
Kim, S.E., Lee, W.J., Choi, K.Y., 2007. The PI3 kinase-Akt pathway mediates Wnt3a-induced proliferation. Cell. Signal. 19, 511–518.
Kim, S.E., Yoon, J.Y., Jeong, W.J., Jeon, S.H., Park, Y., Yoon, J.B., Park, Y.N., Kim, H., Choi, K.Y., 2009. H-Ras is degraded by Wnt/beta-catenin signaling via beta-TrCP-mediated polyubiquitylation. J. Cell Sci. 122, 842–848.
Kinzler, K.W., Vogelstein, B., 1996. Lessons from hereditary colorectal cancer. Cell 87, 159–170.
Lee, J.H., Abraham, S.C., Kim, H.S., Nam, J.H., Choi, C., Lee, M.C., Park, C.S., Juhng, S.W., Rashid, A., Hamilton, S.R., Wu, T.T., 2002. Inverse relationship between APC gene mutation in gastric adenomas and development of adenocarcinoma. Am. J. Pathol. 161, 611–618.
Lewis, A., Segditsas, S., Deheragoda, M., Pollard, P., Jeffery, R., Nye, E., Lockstone, H., Davis, H., Clark, S., Stamp, G., Poulsom, R., Wright, N., Tomlinson, I., 2010. Severe polyposis in Apc(1322T) mice is associated with submaximal Wnt signalling and increased expression of the stem cell marker Lgr5. Gut 59, 1680–1686.
Lievre, A., Bachet, J.B., Boige, V., Cayre, A., Le Corre, D., Buc, E., Ychou, M., Bouche, O., Landi, B., Louvet, C., Andre, T., Bibeau, F., Diebold, M.D., Rougier, P., Ducreux, M., Tomasic, G., Emile, J.F., Penault-Llorca, F., Laurent-Puig, P., 2008. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J. Clin. Oncol. 26, 374–379.
Liu, C., Li, Y., Semenov, M., Han, C., Baeg, G.H., Tan, Y., Zhang, Z., Lin, X., He, X., 2002. Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell 108, 837–847.
MacDonald, B.T., Tamai, K., He, X., 2009. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev. Cell 17, 9–26.
Mann, B., Gelos, M., Siedow, A., Hanski, M., Gratchev, A., Ilyas, M., Bodmer, W., Moyer, M., Riecken, E., Buhr, H., 1999. Target genes of β-catenin–T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. Proc. Natl. Acad. Sci. 96, 1603–1608.
Marshall, C.J., 1995. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. Cell 80, 179–185.
Mendelsohn, J., Baselga, J., 2000. The EGF receptor family as targets for cancer therapy. Oncogene 19, 6550–6565.
Miyoshi, Y., Iwao, K., Nagasawa, Y., Aihara, T., Sasaki, Y., Imaoka, S., Murata, M., Shimano, T., Nakamura, Y., 1998. Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. Canc. Res. 58, 2524–2527.
Moon, B.-S., Jeong, W.-J., Park, J., Kim, T.I., Min, D.S., Choi, K.-Y., 2014. Role of oncogenic K-Ras in cancer stem cell activation by aberrant Wnt/β-catenin signaling. J. Natl. Canc. Inst. 106 djt373.
Morin, P.J., 1999. beta-catenin signaling and cancer. Bioessays 21, 1021–1030.
Park, K.-S., Jeon, S.H., Kim, S.-E., Bahk, Y.-Y., Holmen, S.L., Williams, B.O., Chung, K.-C., Surh, Y.-J., Choi, K.-Y., 2006. APC inhibits ERK pathway activation and cellular proliferation induced by RAS. J. Cell Sci. 119, 819–827.
Phelps, R.A., Chidester, S., Dehghanizadeh, S., Phelps, J., Sandoval, I.T., Rai, K., Broadbent, T., Sarkar, S., Burt, R.W., Jones, D.A., 2009. A two-step model for colon adenoma initiation and progression caused by APC loss. Cell 137, 623–634.
Polakis, P., 1999. The oncogenic activation of beta-catenin. Curr. Opin. Genet. Dev. 9, 15–21.
Polakis, P., 2000. Wnt signaling and cancer. Gene Dev. 14, 1837–1851.
Porter, A.C., Vaillancourt, R.R., 1998. Tyrosine kinase receptor-activated signal transduction pathways which lead to oncogenesis. Oncogene 17.
Radinsky, R., Risin, S., Fan, D., Dong, Z., Bielenberg, D., Bucana, C.D., Fidler, I.J., 1995. Level and function of epidermal growth factor receptor predict the metastatic potential of human colon carcinoma cells. Clin. Canc. Res. 1, 19–31.
Reuter, C.W., Morgan, M.A., Bergmann, L., 2000. Targeting the Ras signaling pathway: a rational, mechanism-based treatment for hematologic malignancies? Blood 96, 1655–1669.
Rodriguez-Viciana, P., Warne, P.H., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, M.J., Waterfield, M.D., Downward, J., 1994. Phosphatidylinositol-3-OH kinase direct target of Ras. Nature 370, 527–532.
Schlessinger, J., 1993. How receptor tyrosine kinases activate Ras. Trends Biochem. Sci. 18, 273–275.
Seshagiri, S., Stawiski, E.W., Durinck, S., Modrusan, Z., Storm, E.E., Conboy, C.B., Chaudhuri, S., Guan, Y., Janakiraman, V., Jaiswal, B.S., Guillory, J., Ha, C., Dijkgraaf, G.J., Stinson, J., Gnad, F., Huntley, M.A., Degenhardt, J.D., Haverty, P.M., Bourgon, R., Wang, W., Koeppen, H., Gentleman, R., Starr, T.K., Zhang, Z., Largaespada, D.A., Wu, T.D., de Sauvage, F.J., 2012. Recurrent R-spondin fusions in colon cancer. Nature 488, 660–664.
Tamai, K., Semenov, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hess, F., Saint-Jeannet, J.-P., He, X., 2000. LDL-receptor-related proteins in Wnt signal transduction. Nature 407, 530–535.
Tan, X., Apte, U., Micsenyi, A., Kotsagrelos, E., Luo, J.H., Ranganathan, S., Monga, D.K., Bell, A., Michalopoulos, G.K., Monga, S.P., 2005. Epidermal growth factor receptor: a novel target of the Wnt/beta-catenin pathway in liver. Gastroenterology 129, 285–302.
Tetsu, O., McCormick, F., 1999. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature 398, 422–426.
Van Aelst, L., White, M., Wigler, M., 1994. Ras Partners. Paper Presented at: Cold Spring Harbor Symposia on Quantitative Biology.
Wheeler, D.L., Huang, S., Kruser, T.J., Nechrebecki, M.M., Armstrong, E.A., Benavente, S., Gondi, V., Hsu, K.T., Harari, P.M., 2008. Mechanisms of acquired resistance to cetuximab: role of HER (ErbB) family members. Oncogene 27, 3944–3956.
Wielenga, V.J., Smits, R., Korinek, V., Smit, L., Kielman, M., Fodde, R., Clevers, H., Pals, S.T., 1999. Expression of CD44 in Apc and TcfMutant mice implies regulation by the WNT pathway. Am. J. Pathol. 154, 515–523.
Yan, Y., Zuo, X., Wei, D., 2015. Concise review: emerging role of CD44 in cancer stem cells: a promising biomarker and therapeutic target. Stem Cells Transl. Med. 4, 1033–1043.
Ying, Y., Tao, Q., 2009. Epigenetic disruption of the WNT/beta-catenin signaling pathway in human cancers. Epigenetics 4, 307–312.
Yonesaka, K., Zejnullahu, K., Okamoto, I., Satoh, T., Cappuzzo, F., Souglakos, J., Ercan, D., Rogers, A., Roncalli, M., Takeda, M., 2011. Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. Sci. Transl. Med. 3 99ra86-99ra86.
Yuasa, Y., 2003. Control of gut differentiation and intestinal-type gastric carcinogenesis. Nat. Rev. Canc. 3, 592–600.
Yun, M.-S., Kim, S.-E., Jeon, S.H., Lee, J.-S., Choi, K.-Y., 2005. Both ERK and Wnt/beta-catenin pathways are involved in Wnt3a-induced proliferation. J. Cell Sci. 118, 313–322.
Zhan, T., Rindtorff, N., Boutros, M., 2017. Wnt signaling in cancer. Oncogene 36, 1461–1473.
Zhang, X., Gaspard, J.P., Chung, D.C., 2001. Regulation of vascular endothelial growth factor by the Wnt and K-ras pathways in colonic neoplasia. Canc. Res. 61, 6050–6054.