

Review

## The Role of Canonical WNT Signaling Pathway in Oral Carcinogenesis: A Comprehensive Review

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**Abstract.** Oral cancer is a common neoplasm worldwide. The incidence and mortality have also increased in recent decades. It is characterized by poor prognosis and a low survival rate despite sophisticated surgical and radiotherapeutic modalities. The WNTs comprise a large family of highly conserved growth factors associated with a number of functions. In this review, we focus largely on the canonical pathway, revealing the recent findings, in oral cancer research, thereby raising our understanding of the mechanisms of this crucial signaling in several cellular activities.

Oral squamous cell carcinoma (OSCC) is a common neoplasm worldwide (1) and it is the most frequent malignant tumor of the oral cavity (2). The incidence and mortality have also increased in recent decades in Europe and USA (3). OSCC is characterized by poor prognosis and a low survival rate (1), despite sophisticated surgical and radiotherapeutic modalities (4). The leading cause of death is metastasis, which occurs primarily by the lymphatic route and whose incidence is significantly correlated with the clinical stage and the localization of primary tumors (5).

Signaling pathways are an ever present force in mammalian cells (6). The WNT signaling pathways play a important role in the differentiation, proliferation, death and function of many cell types and are involved in critical development, growth and homeostatic processes in animals

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(7). The WNTs comprise a large family of highly conserved growth factors associated with a number of functions and pathways (6). In vertebrates, 19 WNT genes are known, these genes participate, as far as is currently known, in four signaling pathways: the Wnt/ $\beta$ -catenin or canonical pathway, a planar cell polarity pathway, the  $Ca^{+2}$ /protein kinase A pathway, and the pathway involving protein kinase C (PKC) in muscle myogenesis (6, 8-10). Regulation of these signaling pathways is crucial; more or less activity bestowed on a signal transduction pathway can cause damage in development, or diseases in adult animals. The best studied of the pathways is the so-called canonical WNT pathway, or the WNT/beta-catenin signaling pathway. In this pathway, there is cytoplasmic accumulation of  $\beta$ -catenin and its subsequent nuclear translocation and activity (7, 11-12). Via binding of WNT to a co-receptor comprised of frizzled and either Lrp5 or Lrp6 in vertebrates.  $\beta$ -catenin levels inside the cell are tightly regulated and normally kept very low by the action of a degradation of axin, adenomatous polyposis coli (APC) and glycogen synthase kinase 3 $\beta$  (GSK-3). When  $\beta$ -catenin accumulates in the cytoplasm, some of it will be translocated into the nucleus where it binds to the TCF/LEF family of transcription factors thereby regulating gene expression (7, 13-15).

In this review, we focus largely on the canonical pathway, revealing the recent findings in oral cancer research, thereby raising our understanding of the mechanisms of this crucial signaling in a several cellular activities.

### WNT Signaling and Apoptosis

The term apoptosis ‘a-po-toe-sis’ was first used in a now-classic paper by Kerr, Wyllie and Currie in 1972 to describe a morphologically distinct form of cell death, although

certain components of the apoptosis concept had been explicitly described many years previously (16, 17). Since apoptosis is so critical for both development and maintenance of healthy tissue, dysregulation of this process can be a contributing factor in many types of diseases such as autoimmune and neurodegenerative diseases and cancer. Cancer can promote alterations of the apoptotic process leading to the inactivation of proapoptotic genes and increased levels of antiapoptotic gene products, giving the neoplastic cells the chance to grow without control (18).

BCL-2 family proteins are important effector genes during the process of apoptosis and are involved in the process of embryogenesis. It has been suggested that the BCL-2 dependent pathway is perhaps the only pathway for apoptosis that is essential for developmentally programmed cell death (as opposed to the pathway that relies on death adaptors) (19-21).

The BCL-2 proto oncogene was originally discovered by the analysis of the t(14;18) chromosomal translocation associated with human follicular B-cell lymphoma (22). It is the most important gene of the BCL-2 family and has been shown to be an inhibitor of apoptosis (23). BAX, another member of the BCL-2 family is considered to be a major effector of apoptosis (24). In normal and tumor tissues, the distribution of BAX is inversely related to that of BCL-2 (25). BCL-2 family proteins have been shown to interact with each other in pathways in the assessment of tumor aggressiveness both in humans and animal models for oral cancer research (26-29). Formation of specific mitochondrial membrane pores occurs through the action of apoptosis-promoting members of the BCL-2 family. These pores are called permeability transition (PT) pores. Once PT pores are formed, pro-apoptotic molecules – AIF, SMAC, DIABLO and cytochrome c exit the mitochondrion. The release of cytochrome c into the cytosol is an especially significant event for the induction of apoptosis. Cytochrome c interacts with APAF (the homologue of CED-4 in *C. elegans*) which in turn activates caspase-9 and later caspase-3. A multiprotein caspase death-inducing signaling complex (apoptosome) forms, comprising cytochrome c, APAF-1, procaspase-9 and ATP (30).

Recent reports in the literature have shown the association between apoptosis and the WNT signaling pathway through a variety of mechanisms (17, 18, 31, 32). Genes in both the apoptotic and WNT pathways are activated in a coordinated manner throughout development (31). The activity of WNT signaling according to specific cellular environment stimuli can either foster or restrain the processes of apoptosis (33-34). WNT signaling regulates the early and late stages of apoptosis in both development and cellular injury in the cell populations of neurons, endothelial cells, vascular smooth muscle cells and cardiomyocytes (35).

There are some mechanisms related to WNT signaling and apoptosis pathways. These include WNT-BMP signaling,

through SFRP2 (secreted Frizzled-related protein-2), gene expression, through  $\beta$ -catenin, GSK-3 $\beta$ -NF $\kappa$ Beta, c-JUN N-terminal kinase signaling or gene expression of DICKKOPF-1, nemo, sox 10 and tau (35-37).

GSK-3 $\beta$  is an AKT substrate shown to be inhibited upon phosphorylation by AKT (38). GSK-3 $\beta$  triggers the degradation and inactivation of several oncogenic transcription factors (*e.g.*, c-jun and c-myc) and proto-oncoproteins (*e.g.*,  $\beta$ -catenin) by phosphorylating them and thus it is expected to suppress neoplastic transformation and tumor development (39). GSK-3 $\beta$  is also a negative regulator of the WNT- $\beta$ -catenin pathway (40). Studies have demonstrated that mitochondrial damage plays an important role in initiating the cascade of caspase activation in response to stress stimuli, such as chemotherapeutic drugs (41, 42). Disruption of the mitochondrial membrane by apoptotic stimuli results in the release of cytochrome c into the cytosol (43). Chen *et al.* (44) found that WNT/ $\beta$ -catenin signaling inhibited mitochondria-dependent apoptosis in colorectal cancer cells (44). Cytochrome c along with ATP and APAF-1, recruits and processes procaspase-9 (45, 46). Active caspase-9 subsequently activates effector caspases, such as caspase-3, which in turn cleave key proteins to induce apoptosis (43, 46). Although it is not known whether WNT signaling modulates the cell death machinery, interestingly, overexpression of several downstream mediators of WNT signaling has been found to regulate apoptosis (47-50).

Tumor necrosis factor (TNF) is one of the primary inducers for death receptor-mediated apoptosis. TNF- $\alpha$  is a founding member of the TNF super-family of proteins. It is well known that TNF-induced cytotoxicity is enhanced in the presence of either a protein synthesis inhibitor or a transcription inhibitor (51, 52). When activation of c-myc renders cells susceptible to TNF-induced apoptosis, the WNT/ $\beta$ -catenin signaling pathway has been shown to be capable of acting against c-Myc and inhibits TNF/c-myc mediated apoptosis, indicating that WNT signaling may have a survival function in cancer cells (53).

WNT proteins bind to receptors of the frizzled family on the cell surface through several cytoplasmic relay components, the signal is transduced to  $\beta$ -catenin, which enters the nucleus to activate transcription of WNT target genes. APC is a critical component in the formation of multiprotein complex ligand with axin/axin2, casein kinase and GSK-3 $\beta$ .  $\beta$ -Catenin is recruited to this complex, phosphorylated ubiquitinated and is consequently moved to the proteasome. When this WNT ligand is absent,  $\beta$ -catenin is continually destroyed. In response to WNT signaling, or when APC is  $\beta$ -catenin stabilized, accumulates in the cytoplasm and enters the nucleus. Together, members of the LEF/TCF family with the transcription of target genes, such c-Myc and cyclin D1, related to carcinogenesis is activated (7, 12, 44, 54, 55).

Interestingly, APC is a suppressor protein that participates in the apoptosis process (34, 55, 56). APC is illustrative of the multiple roles that certain tumor suppressors play in the cell, being many cellular functions: as a component of the WNT signal transduction pathway, of adherens junctions and the mechanism of cytoskeleton stabilization. Mutation analysis of the APC gene revealed over 400 different germline mutations responsible for familial adenomatous polyposis coli (FAP) (54). The role of APC in apoptosis regulation is dependent on whether it comprises of full length (wild-type), or truncated (mutant) proteins. Overexpression of wild-type APC induces apoptosis, while overexpression of mutant truncated proteins maintains an anti-apoptotic mode of action (54). These mechanisms represent exceptional potential targets for treatments of several diseases.

### WNT Signaling and Oral Cancer

WNT signaling has been identified as one of the key signaling pathways in cancer, regulating cell growth, motility and differentiation. Because of its widespread activation in diverse types of human tumor, the WNT pathway has gained considerable and growing interest in tumor research over recent years.

Recently, several components of the WNT pathway, in particular those involved in degradation of  $\beta$ -catenin, were shown to be associated with microtubules and the mitotic spindle apparatus (57). It appears that the members of the  $\beta$ -catenin destruction are complex, whether this reflects a new functional property of the complex related to the WNT pathway remains to be analyzed. Indeed, localization of these components might have important consequences for cancer development.

Several studies have demonstrated that aberrant activation of the WNT signaling pathway contributes to neoplastic transformation in oral tissues (58, 59). The WNT family participates in epithelial cell proliferation and the aberrant activation of this pathway may contribute to cancer progression (60). Whereas normal oral epithelium exhibits exclusively  $\beta$ -catenin immunostaining (61), mutations of  $\beta$ -catenin resulting in forming a complex in the nucleus TCF/LEF could be not detected in immunohistochemical studies (61). OSCC cells leak membrane-bound  $\beta$ -catenin revealing a corresponding increase in cytoplasmic localization (13) and sporadic nuclear accumulation (13, 62).

Studies have shown membrane expression of  $\beta$ -catenin (63, 64), correlated with differentiation and significantly associated with invasion and poor prognosis (65, 66). Nevertheless, Kildal postulated that cytoplasmic and membranous  $\beta$ -catenin staining were not of prognostic importance (67); indeed nuclear  $\beta$ -catenin staining was associated with improved

survival and occurred preferentially in endometrial carcinoma, indicating nuclear  $\beta$ -catenin expression as indicates good prognosis in contrast to patients who expressed only membranous  $\beta$ -catenin (67, 68). Using an experimental model with 4-nitroquinoline 1-oxide to induce tongue cancer, our group also demonstrated an overexpression of  $\beta$ -catenin in the cytoplasm of the negative control and those exposed to the carcinogen. Probably,  $\beta$ -catenin can be inhibited by non-canonical pathways through diverse mechanisms, such as activation of PKC and/or Siah2 (69). Taken as a whole,  $\beta$ -catenin plays a crucial role in human tumorigenesis (70).

Mutations of APC have been widely identified in colonic cancer, esophageal (71), and gastric cancer (72). Iwai *et al.* found a polymorphism of the APC gene within exon 15 in an OSSC line, this mutation as well in axin (59) can cause constitutive signaling independent of the upstream signal from WNT. Previous studies have shown that APC and axin are frequently mutated in different types of human cancer (73). No mutations of the  $\beta$ -catenin gene have been found in primary esophageal cancer (74). According studies in OSCC, mutations were rarely found in OSCC (75).

It seems that WNT-1 appears to play an important role in basal cell carcinomas. WNT-1 was first identified as a mammary oncogene activated by mouse mammary tumour virus (MMTV) (76); it contributes to mammary oncogenesis when overexpressed (76, 77). WNT-1 is responsible for a decrease in the activity of GSK-3  $\beta$  and an increase in the  $\beta$ -catenin pool, modulating cadherin catenin complex formation and cell-to-cell adhesion, and activating tyrosine kinase receptors that can rescue cells from apoptosis (78). It has been suggested that the WNT-1 pathway may participate in oral carcinogenesis (79). In addition, this pathway appears to play an important role in basal cell carcinomas as for, example in areas of infiltration and/or of focal dedifferentiation of basal cell carcinoma (14). Studies by Fracalossi *et al.* showed WNT-1 to be overexpressed in the cytoplasm of the majority of carcinogenesis experimental models using 4NQO at 12 and 20 weeks (15). It is suggested that the expression and function of some WNT genes as a result of increase in mRNA levels from head and neck OSCC line. Moreover, the treatment of OSCC line with anti-WNT-1 antibodies reduced the activity of WNT/frizzled-dependent transcription factor TCF/LEF and diminished the expression of cyclin D1 and  $\beta$ -catenin proteins (80, 81). Blocking WNT-1 signaling inhibited proliferation and induced apoptosis. Taken together, the role of the WNT-1 pathway in oral carcinogenesis aids on in understanding the relationship between adhesion molecules, transcriptional factors and potent oncogenes (15).

The role of frizzled in acting as a receptor for WNTs is long established; frizzled carries an extracellular cysteine-rich domain that is sufficient to bind WNT proteins and the addition of frizzled to WNT cells non-responsive to WNT

can render signaling competence (82). With regard to frizzled proteins, Fractalossi *et al.* suggested that the frizzled proteins are not involved in the signaling WNT pathway when carcinogenesis experimental model was used (15). Prado *et al.* observed no differences in frizzled-5 expression in head and neck SCC cell lines against normal oral squamous epithelial cell lines. However, in a study of head and neck SCC tissues an increased expression of frizzled-5 was related when compared to healthy human oral mucosa (83). Aberrant activation of WNT and frizzled signaling pathway has been reported in OSCC line (80). It is crucial to stress that the complexity of the WNT pathway extends to its receptors. When the interaction of co-receptor LRP5/6 and frizzled-1 is considered, the transmission of the canonical WNT signaling occurs as expected. However, the interaction of the co-receptor LRP 1 with frizzled-1 represses canonical signaling (84, 85).

Interest in the WNT signaling pathway continues. WNTs have been mentioned in 5089 journal articles to date; however only 31 regarding WNT and oral squamous cell carcinoma were listed on PubMed in 11 years. For these reasons, further studies should be carried out to better understand their relation to carcinogenesis process.

## Conclusion

The WNT pathway plays an important role in development, growth and maintenance of cells, tissues and organisms. Understanding WNT signaling in oral cancer and how this pathway directs cellular behavior will aid in the development of new small molecular and pharmaceutical agents to treat these and several other diseases.

## References

- Magrath I and Litvak J: Cancer in developing countries: opportunity and challenge. *J Nat Cancer Inst* 85: 862-874, 1993.
- Lese C, Rossie K, Appel B, Reddy J, Johnson I and Myers E: Visualization of *INT2* and *HST1* amplification in oral squamous cell carcinoma. *Genes Chromosomes Cancer* 12: 288-295, 1995.
- Rodrigues V, Moss S and Tuomainen H: Oral cancer in the UK to screen or not to screen?. *Oral Oncol* 34: 454-465, 1998.
- Xi S and Grandis J: Gene therapy for the treatment of oral squamous cell carcinoma. *J Dent Res* 82: 11-16, 2003
- Berenson J, Yang I and Mickel R: Frequent amplification of the *BCL-1* locus in head and neck squamous cell carcinomas. *Oncogene* 4: 1111-1116, 1989.
- Logan C and Nusse R: The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 20: 781-810, 2004.
- Johnson M, and Rajamannan N: Diseases of Wnt signaling. *Rev Endocr Metab Disord* 7: 41-49, 2006.
- Mlodzik M: Planar cell polarization: Do the same mechanisms regulate drosophila tissue polarity and vertebrate gastrulation? *Trends Genet* 18: 564-571, 2002.
- Kuhl M, Sheldahl L, Park M, Miller J and Moon R: The Wnt/Ca<sup>2+</sup> pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet* 16: 279-283, 2000.
- Chen A, Ginty D and Fan C: Protein kinase a signalling via CREB controls myogenesis induced by Wnt proteins. *Nature* 433: 317-322, 2005.
- Muzio LL, Goteri G and Capretti R: Beta-catenin gene analysis in oral squamous cell carcinoma. *Int J Immunopathol Pharmacol* 18: 33-38, 2005.
- Gordon M and Nusse R: Wnt signaling: multiple pathways, multiple receptors and multiple transcription factors. *J Biol Chem* 281: 22429-22433, 2006.
- Muzio LL, Staibano S, Pannone G, Grieco M, Mignani M, Cerrato A, Testa N and Rosa GD:  $\beta$ - and  $\gamma$ -catenin expression in oral squamous cell carcinomas. *Anticancer Res* 19: 3817-3826, 1999.
- Muzio ML: WNT-1 expression in basal cell carcinoma of head and neck. An immunohistochemical and confocal study with regard to the intracellular distribution of  $\beta$ -catenin. *AntiCancer Res* 22: 565-576, 2002.
- Fractalossi A, Silva M, Oshima C and Ribeiro D: Wnt/ $\beta$ -catenin signalling pathway following rat tongue carcinogenesis induced by 4-nitroquinoline 1-oxide. *Exp Mol Pathol* 88: 176-183, 2010.
- Kerr J, Wyllie A and Currie A: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26: 239-257, 1972.
- Kerr J: History of the events leading to the formulation of the apoptosis concept. *Toxicology* 181-182: 471-474, 2002.
- Evan G and Vousden K: Proliferation, cell cycle and apoptosis in cancer. *Nature* 411: 342-348, 2001.
- Cecconi F, Alvarez-Bolado G, Meyer B, Roth K and Gruss P: Apaf1 (CED-4 homolog) regulates programmed cell death in mammalian development. *Cell* 94: 727-737, 1998.
- Yoshida H, Kong Y, Yoshida R, Elia A, Hakem A, Hakem R, Penninger J and Mak T: Apaf1 is required for mitochondrial pathways of apoptosis and brain development. *Cell* 94: 739-750, 1998.
- Aravind L, Dixit V and Koonin E: The domains of death: Evolution of the apoptosis machinery. *Trends Biochem Sci* 24: 47-53, 1999.
- Tsujimoto Y, Jaffe E, Cossman J, Gorham J, Nowell P and Croce C: Clustering of breaking points on chromosome 11 in human B-cell neoplasms with the t(11,14) translocation. *Nature* 315: 340-343, 1985.
- Lu Q, Abel P, Foster C and Lalani E: BCL-2: role in epithelial differentiation and oncogenesis. *Hum Pathol* 27: 102-110, 1996.
- Oltvai Z, Millman C and Korsmeyer S: Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 74: 609-619, 1993.
- Krajewska M, Fenoglio-Preiser C, Krajewski S, Song K, Macdonald J, Stemmerman G and Reed J: Immunohistochemical analysis of BCL-2 family proteins in adenocarcinomas of the stomach. *Am j Pathol* 149: 1449-1457, 1996.
- Yao L, Iwai M and Furuta I: Correlations of BCL-2 and p53 expression with the clinicopathological features in tongue squamous cell carcinomas. *Oral Oncology* 35: 56-62, 1999.
- Nishimura A: Changes in Bcl-2 and Bax expression in rat tongue during 4-nitroquinoline 1-oxide-induced carcinogenesis. *J Dent Res* 78: 1264-1269, 1999.

- 28 Ribeiro D, Salvadori D and Marques M: Abnormal expression of Bcl-2 and Bax in rat tongue mucosa during the development of squamous cell carcinoma induced by 4-nitroquinoline 1-oxide. *Int J Exp Pathol* 86: 375-381, 2005.
- 29 Minicucci E, Ribeiro D, Silva GN, Pardini M, Montovani J and Salvadori D: The role of the TP53 gene during rat tongue carcinogenesis induced by 4-nitroquinoline 1-oxide. *Exp Toxicol Pathol* 63: 483-489, 2011.
- 30 Dash P: <http://www.sgulacuk/depts/immunology/~dash/apoptosis/2008>.
- 31 Simonis M, Klous P, Splinter E, Moshkin Y, Willemsen R, Wit ED, Steensel BV and Laat WD: Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). *Nat Genet* 38: 1348-1354, 2006.
- 32 Kaur J and Sanyal S: PI3-kinase/Wnt association mediates COX-2/PGE-2 pathway to inhibit apoptosis in early stages of colon carcinogenesis: chemoprevention by diclofenac. *Tumor Biol* 31: 623-631, 2010.
- 33 Yeo W and Gautier J: Early neural cell death: dying to become neurons. *Dev Biol* 274: 233-244, 2004.
- 34 Brocardo M and Henderson B: APC shuttling to the membrane, nucleus and beyond. *Trends Cell Biol* 18: 587-596, 2008.
- 35 Li F, Chong Z and Maiese K: Winding through the WNT pathway during cellular development and demise. *Histol Histopathol* 21: 103-124, 2006.
- 36 Crowder R and Freeman R: Glycogen synthase kinase-3 beta activity is critical for neuronal death caused by inhibiting phosphatidylinositol 3- kinase or Akt but not for death caused by nerve growth factor withdrawal. *J Biol Chem* 275: 34266-34271, 2000.
- 37 Ellies D, Church V, Francis-West P and Lumsden A: The WNT antagonist cSFRP2 modulates programmed cell death in the developing hindbrain. *Development* 127: 5285-5295, 2000.
- 38 Zheng W, Kar S and Quirion R: Insulin-like growth factor-1-induced phosphorylation of the forkhead family transcription factor FKHRL1 is mediated by AKT kinase in PC12 cells. *J Biol Chem* 275: 39152-39158, 2000.
- 39 Manoukian A and Woodgett J: Role of glycogen synthase kinase 3 in cancer: regulation by Wnts and other signaling pathways. *Adv Cancer Res* 84: 203-229, 2002.
- 40 Shakoori A, Ougolkov A, Yu Z, Zhang B, Modarressi M and Billadeau D: Deregulated GSK3 $\beta$  activity in colorectal cancer: its association with tumor cell survival and proliferation. *Biochem Biophys Res Commun* 334: 1365-1373, 2005.
- 41 Chao DT and Korsmeyer SJ: BCL-2 family: regulators of cell death. *Annu Rev Immunol* 16: 395-419, 1998.
- 42 Green D and Reed J: Mitochondria and apoptosis. *Science* 281: 1309-1312, 1998.
- 43 Liu X, Kim C, Yang J, Jemmerson R and Wang X: Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome *c*. *Cell* 86: 147-157, 1996.
- 44 Chen S, Guttridge D, You Z, Zhang Z, Fribley A, Mayo M, Kitajewski J and Wang C: Wnt-1 signaling inhibits apoptosis by activating beta-catenin/T cell factor-mediated transcription. *J Cell Biol* 152: 87-96, 2001.
- 45 Li P, Nijhawan D, Budihardjo I, Srinivasula S, Ahmad M, Alnemri E and Wang X: Cytochrome *c* and dATP-dependent formation of Apaf-1/ caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91: 479-489, 1996.
- 46 Zou H, Henzel W, Liu X, Lutschg A and Wang X: Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome *c*-dependent activation of caspase-3. *Cell* 90: 405-413, 1997.
- 47 Morin P, Vogelstein B and Kinzler K: Apoptosis and APC in colorectal tumorigenesis. *Proc Natl Acad Sci USA* 93: 7950-7954, 1996.
- 48 TC He, Chan T, Vogelstein B and Kinzler K: PPAR is an APC regulated target of nonsteroidal anti-inflammatory drugs. *Cell* 99: 335-345.
- 49 Orford K, Orford C and Byers S: Exogenous expression of beta-catenin regulates contact inhibition, anchorage-independent growth, anoikis, and radiation-induced cell cycle arrest. *J Cell Biol* 146: 855-886, 1999.
- 50 Strovel E and Sussman D: Transient overexpression of murine dishevelled genes results in apoptotic cell death. *Exp Cell Res* 253: 637-648, 1999.
- 51 Johnson B, Cepero E and Boise L: Bcl-xL inhibits cytochrome *c* release but not mitochondrial depolarization during the activation of multiple death pathways by tumor necrosis factor-alpha. *J Biol Chem* 275: 31546-31553, 2000.
- 52 Zhang S, Kovalenko A, Cantarella G and Wallach D: Recruitment of the IKK signalosome to the p55 TNF receptor: RIP and A20 bind to NEMO (IKKgamma) upon receptor stimulation. *Immunity* 12: 301, 2000.
- 53 You Z, Saims D, Chen S, Zhang Z, Guttridge D, Guan K, MacDougald O, Brown A, Evan G, Kitajewski J and Wang C: Wnt signaling promotes oncogenic transformation by inhibiting c-Myc-induced apoptosis. *J Cell Biol* 29: 429-440, 2002.
- 54 Pećina-Šlaus N, Majić Z, Musani V, Zeljko M and Xupić H: Report on mutation in exon 15 of the APC gene in a case of brain metastasis. *Neurooncol* 97: 143-148, 2010.
- 55 Chen T, Yang I, Irby R, Shain K, Wang H, Quackenbush J, Coppola D, Cheng J and Yeatman T: Regulation of caspase expression and apoptosis by adenomatous polyposis coli. *Cancer Res* 63: 4368-4374, 2003.
- 56 Hasegawa S, Sato T, Akazawa H, Okada H, Maeno A, Ito M, Sugitani Y, Shibata H, Miyazaki J, Katsuki M, Yamauchi Y, Yamamura K, Katamine S and Noda T: Apoptosis in neural crest cells by functional loss of APC tumor suppressor gene. *Proc Natl Acad Sci USA* 99: 297-302, 2002.
- 57 Mogensen M, Tucker J, Mackie J, Prescott A and Nathke I: The adenomatous polyposis coli protein unambiguously localizes to microtubule plus ends and is involved establishing parallel arrays of microtubules bundles in highly polarized epithelial cells. *J Cell Biol* 157: 1041-1048, 2002.
- 58 Uraguchi M, Morikawa M, Shirakawa M, Sanada K and Imai K: Activation of WNT family expression and signaling in squamous cell carcinomas of the oral cavity. *J Dent Res* 83: 327-332, 2004.
- 59 Iwai S, Katagiri W, Amekawa C, Nakazawa M and Yura Y: Mutations of the APC, beta-catenin, and axin 1 genes and cytoplasmic accumulation of beta-catenin in oral squamous cell carcinoma. *J Cancer Res Clin Oncol* 131: 773-782, 2005.
- 60 El-Bahrawy M, El-Masry N, Alison M, Poulson R and Fallowfield M: Expression of beta-catenin in basal cell carcinoma. *Br J Dermatol* 148: 964-970, 2003.
- 61 Pannone G, Muzio LL, Bucci P, Canfora M, Pannone G, Santacroce L, Bucci T and Staibano S: Physiopathology of  $\beta$  and  $\chi$ -catenin expression in the oral epithelium. *Minerva Stomatol* 47: 583-588, 1998.

- 62 Lyakhovitsky A, Barzilai A, Fogel M, Trau H and Huszar M: Expression of E-cadherin and  $\beta$ -catenin in cutaneous squamous cell carcinoma and its precursors. *Am J Dermatopathol* 26: 372-378, 2004.
- 63 Laxmidevi L, Angadi P, Pillai R and Chandreshekar C: Aberrant  $\beta$ -catenin expression in the histologic differentiation of oral squamous cell carcinoma and verrucous carcinoma: an immunohistochemical study. *J Oral Sci* 52(4): 633-640, 2010.
- 64 Schussel J, Jr DP and Martins M: Altered  $\beta$ -catenin expression related to cancer progression on actinic cheilitis and squamous cell carcinoma of the lip. *Ann Diag Pathol* 15: 1-5, 2011.
- 65 Mahomed F, Altini M and Meer S: Altered E-cadherin/ $\beta$ -catenin expression in oral squamous carcinoma with and without nodal metastasis. *Oral Dis* 13: 386-392, 2007.
- 66 Cai Z, Shi X, Gao Y, Wei M, Wang C and Yu G:  $\beta$ -catenin expression pattern in primary oral squamous cell carcinoma. *Chin Med J* 121: 1866-1870, 2008.
- 67 Kildal A:  $\beta$ -catenin expression, DNA ploidy and clinicopathological features in ovarian cancer, a study in 253 patients. *Eur J Cancer* 41: 1127-1134, 2005.
- 68 Gamallo C, Palacios J, Moreno G, Mora JCD, Suárez A and Armas A:  $\beta$ -catenin expression pattern in stage I and II ovarian carcinomas, relationship with  $\beta$ -catenin gene mutations, clinicopathological features, and clinical outcome. *Am J Pathol* 155: 527-536, 1999.
- 69 Topol L, Jiang X, Choi H, Garrett-Beal L, Carolan P and Yang Y: Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent  $\beta$ -catenin degradation. *J Cell Biol* 162: 899-908, 2003.
- 70 Davies B, Worsley S and Ponder B: Expression of E-cadherin,  $\alpha$ -catenin and  $\beta$ -catenin in normal ovarian surface epithelium and epithelial ovarian cancers. *Histopathology* 32: 69-80, 1998.
- 71 Boynton R, Blount P, Yin J, Brown V, Huang Y, Tong Y, McDaniel T, Newkirk C, Resau J, Raskind W, Haggitt R, Reid B and Meltzer S: Loss of heterozygosity involving the APC and MCC genetic loci occurs in the majority of human esophageal cancers. *Proc Natl Acad Sci USA* 89(8): 3385-3388, 1992.
- 72 Miyoshi Y, Nagase H, Ando H, Horii A, Ichii S, Nakatsuru S, Aoki T, Miki T, Mori T and Nakamura Y: Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. *Hum Mol Genet* 1(4): 229-233, 1992.
- 73 Pannone G, Bufo P, Santoro A, Franco R, Aquino F, Longo G, Botti G, Serpico R, Cafarelli B, Abbruzze A, Caraglia M, Papageraki S and LoMuzio L: WNT pathway in oral cancer: Epigenetic inactivation of WNT-inhibitors. *Oncol Rep* 24: 1035-1041, 2010.
- 74 Mir MM, Dar JA, Dar NA, Syed AT, Salam I and Lone GN: The association of  $\beta$ -catenin gene mutations and human papillomavirus in carcinoma of esophagus in a high-risk population of India. *Int J Health Sci (Qassim)* 1: 177-183, 2007.
- 75 Ninomiya I, Endo Y, Fushida S, Sasagawa T, Miyashita T, Fujimura T, Nishimura G, Tani T, Hashimoto T, Yagi M, Shimizu K, Ohta T, Yonemura Y, Inoue M, Sasaki T and Miwa K: Alteration of  $\beta$ -catenin expression in esophageal squamous-cell carcinoma. *Int J Cancer* 85(6): 757-761, 2000.
- 76 Yeh K, Chang J, Lin T, Wang Y, Chang Y, Shih M and Lin C: Correlation between protein expression and epigenetic and mutation changes of Wnt pathway-related genes in oral cancer. *Int J Oncol* 23(4): 1001-1007, 2003.
- 77 Nusse R, Ooyen Av, Cox D, Fung Y and Varmus H: Mode of proviral activation of a putative mammary oncogene (INT-L) on mouse chromosome 15. *Nature* 307: 131-136, 1984.
- 78 Brown A, Wildin R, Prendergast T and Varmus H: A retrovirus vector expressing the putative mammary oncogene INT-L causes partial transformation of a mammary epithelial cell line. *Cell* 46: 1001-1009, 1986.
- 79 Papkoff J, Rubinfeld B, Schryver B and Polakis P: Wnt-1 regulates free pools of catenins and stabilizes APC catenin complexes. *Mol Cell Biol* 16: 2128-2134, 1996.
- 80 Muzio LL: A possible role for the Wnt-1 pathway in oral carcinogenesis. *Crit Rev Oral Biol Med* 12: 152-165, 2001.
- 81 Rhee C: Wnt and frizzled receptors as potential targets for immunotherapy in head and neck squamous cell carcinomas. *Oncogene* 21: 6598-6605, 2002.
- 82 Oshima H, Matsunaga A, Fujimura T, Tsukamoto T, Taketo M, and Oshima M: Carcinogenesis in mouse stomach by simultaneous activation of the Wnt signaling and prostaglandin E2 pathway. *Gastroenterology* 131: 1086-1095, 2006.
- 83 Bahnot O, Brink M, Samos S, Hsieh J, Wang Y, Macke J, Andrew D, Nathans J and Nusse R: A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. *Nature* 382(6588): 225-230, 1996.
- 84 Prado S, Villaamil V, Gallego G, Calvo M, Cedrún J, Soliva S, Ayerbes M, Campelo R and Aparicio L: Expression of Wnt gene family and frizzled receptors in head and neck squamous cell carcinomas. *Virchows Arch* 455: 67-75, 2009.
- 85 Zilberberg A, Yaniv A and Gazit A: The low density lipoprotein receptor-1, LRP1, interacts with the human frizzled-1 (HFz1) and down-regulates the canonical Wnt signaling pathway. *J Biol Chem* 279: 17535-17542, 2004.

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