Review Article

Oral cancer stem cells: Drivers of local recurrence and metastasis

Komal Khot, Swati Deshmane

Department of Oral and Maxillofacial Pathology, Y.M.T. Dental College and PG Institute, Navi Mumbai, Maharashtra, India

ABSTRACT

Oral squamous cell carcinoma (OSCC) is one of the most feared human cancers due to its aggressiveness and high mortality rate. One of the reasons for its treatment failure is thought to be related to the presence of a subpopulation of cells within the tumor called cancer stem cells (CSCs) which are highly tumorogenic, capable of self-renewal, and have the ability to differentiate into cells that constitute the bulk of tumors. Recent evidence suggests that CSCs are especially, resistant to conventional therapy and are the "drivers" of local recurrence and metastatic spread. Research in the recent years has been aimed at the application of stem cell biology to clinical medicine, particularly its role in the evolution and metastasis of tumors. Several stem cell markers are known to be expressed, mainly in the basal layers of oral mucosa which are necessary for tissue homeostasis. These specific markers for CSCs have been investigated in head and neck SCC in the hope of developing a deeper understanding of the role of CSCs in oral cancer pathogenesis, diagnosis, and developing newer therapeutic strategies. This review aims at presenting CSC and their biomarkers with a special emphasis on their role in oral tumorogenesis and OSCC metastasis. It also aims to explain the potential role of these CSC in improving the diagnosis, prognosis, and treatment of OSCC patients.

Key words: Metastasis, oral cancer stem cells, recurrence, stem cells

INTRODUCTION

Under current therapeutics, the prognosis of oral squamous cell carcinoma (OSCC) remains dismal. This is because more than 50% of patients succumb within a short period ranging from a few months to 3–5 years. In recent years, stem cells have been the focus of a tremendous amount of biomedical research because of their apparent potential for regeneration. Several studies have implicated cancer stem cells (CSCs) as an important factor in cancer progression, invasion, metastasis, and recurrence.[1] How the CSC theory fits into the general scheme of cancer progression, in particular to metastasis, has not been well-defined. CSCs may play an important role in tumor metastasis, and their understanding may provide insights into the development of predictive and prognostic markers and specific therapeutic interventions.[2]

This review aims to explain the role of CSCs in both tumorigenesis and metastasis. A better understanding of CSCs as a fundamental component of the metastatic cascade will lead to novel therapeutic strategies against metastatic cancer.

HISTORY

CSCs as defined by American Association of Cancer Research, are a subset of cells with the capability of self-renewal and differentiation into the heterogeneous lineages that constitute the tumor mass.[3] The role of stem cells in cancer has been built up over the last several decades. Due to his contribution in this field almost a century ago, John Beard is considered "father of CSC biology." However, the first direct evidence of the existence of CSCs came from

Address for correspondence: Dr. Swati Deshmane, Karad- Chiplun Road, Ramapur, Patan, Tal-Patan, Satara - 415 206, Maharashtra, India.
E-mail: swatideshmane77@gmail.com

Access this article online

Quick Response Code:

Website: www.ccij-online.org

DOI: 10.4103/2278-0513.168516

Cite this article as: Khot K, Deshmane S. Oral cancer stem cells: Drivers of local recurrence and metastasis. Clin Cancer Investig J 2015;4:691-6.

the work of Bonnet and Dick who identified the presence of CSCs in acute lymphocytic leukemia through extensive cell cloning.[2,4,5] Further studies by Bonnet and Dick, using lineage tracing proved that a single leukemia stem cell could give rise to various populations of leukemia cells.[2,5,6] The first report of CSCs in solid cancer came in 2003 from Al-Hajj *et al*., who demonstrated the presence of CSCs in breast cancer.[7] To date, CSCs have been discovered in a broad spectrum of solid tumors, including lung cancer, brain cancer, colon cancer, ovarian cancer, prostate cancer, and melanoma.[8]

In head and neck tumors, CSC isolation was first performed by Prince and Ailles *et al.* through the expression of CD44 by a few subpopulation of cells.[9]

CANCER STEM CELL ORIGIN

The discovery of CSCs begets the question regarding the origin of these cells. The origin of the cancer-initiating cell has long been presumed to be the normal endogenous tissue stem cell. This is based upon their similar behaviors and the notion that only accumulated mutations within a long-lived cell could ultimately result in tumorigenesis. In colorectal cancer, a strong correlation has been noted between induced loss of the Wnt signaling molecule adenomatous polyposis coli (APC) in a putative stem cell population and the formation of benign intestinal polyps, thus, providing evidence that intestinal cancers can arise from a progenitor population. However, it is possible that accumulation of genetic mutations within a differentiated or progenitor cell can allow expression of stem cell behavior, and that this may provide an alternative source of CSCs.[10] The genetic and epigenetic instability of these cells may result in the accumulation of mutations that enable them to acquire the ability of self-renewal and tumorigenicity. Furthermore, the epithelial-mesenchymal transition (EMT) is also one mechanism which generates CSCs endowed with an invasive and metastatic phenotype. EMT is a series of steps, resulting in the transformation of epithelial cells into fibroblast-like and motile cells, and eventually the cancer cells acquire the ability to invade, migrate, and disseminate.[11]

The key features of the CSC hypothesis were described by Prince and Ailles in 2008:[9]

- Only a small fraction of the cancer cells within a tumor have tumorigenic potential when transplanted into immunodeficient mice
- The CSC subpopulation can be separated from the other cancer cells by distinctive surface markers
- Tumors resulting from the CSCs contain the mixed tumorigenic and nontumorigenic cells of the original tumor
- The CSC subpopulation can be serially transplanted through multiple generations, indicating that it is a self-renewing population.

Role of human papillomavirus

Human papillomavirus (HPV) has been suggested as an etiologic agent for a subset of oral pathogenesis. The HPV-positive cells show expression of early oncogenic proteins E6 and E7 which target the proteins such as p53, pRb, Notch-1, p16, cyclinD1, and EGFR. The study showed that HPV E6 and E7 activated Wnt signaling pathway [Figure 1] in HPV16-positive oropharyngeal squamous carcinoma cells and might be involved in de-differentiation to CSCs from oral cancer cells.[12] Recently, a new HPV positive UM-SCC-104 head and neck squamous cell carcinoma (HNSCC) cell line has been identified that has subpopulation of aldehyde dehydrogenase (ALDH) CSCs which is highly tumorigenic, has ability to self-renew, and has the capacity to recreate tumor heterogeneity.[13]

Tobacco chewing and heavy smoking

Cigarette smoking and tobacco chewing are widely considered to be the major risk factors for the development of oral cancer. Nicotine is a major component of tobacco and hampers cellular functions. Long-term exposure to nicotine was demonstrated to up-regulate ALDH1 population in normal gingival and primary OSCC oral epithelial (OE) cells. It enhanced the self-renewal sphere-forming ability, stemness gene signatures expression, and EMT regulators in OE cells.[14] Furthermore, cigarette smoke is associated with drug resistance in OCSCs. A significant association

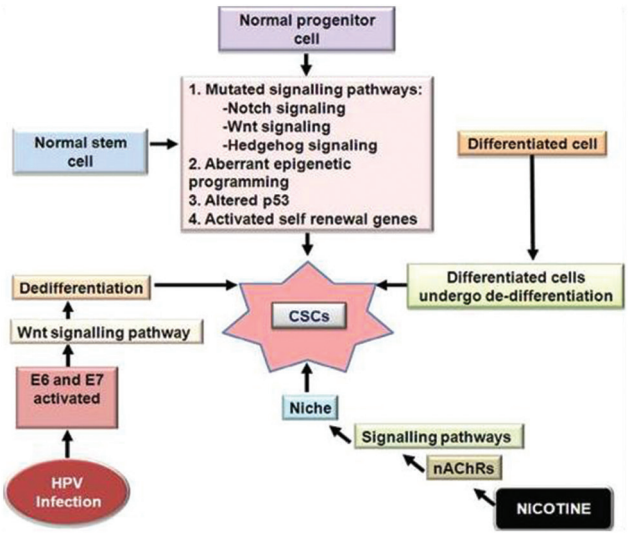

Figure 1: Possible origin of oral cancer stem cells

between CD44 expression and heavy smoking and/or alcohol consumption which predicts poor prognosis has been observed in oral cancer.[13]

CANCER STEM CELL BIOMARKERS

Due to the close relationship between CSCs and tumor initiation, progression, metastasis, and drug resistance, the isolation of these cells from the cancer cell population is essential for detailed studies. Specific surface biomarker phenotypes can be used to distinguish CSCs from other tumor cells and normal stem cells. Currently, the most common method used to identify CSCs is fluorescence-activated cell sorting based on cell surface markers or intracellular molecules.[8] Molecular markers implicated in HNSCC CSC detection are given in Table 1.

KEY SIGNALING PATHWAYS

Dysregulation of signaling pathway networks enables CSCs to retain stem cell properties. As shown in Figure 2, the pathways and elements that are involved in the control of self-renewal and differentiation of CSCs and normal stem cells include PI3K/Akt, PTEN, JAK/STAT, Wnt/β-catenin, hedgehog (Hh), Notch, NF-κB, and Bcl-2.

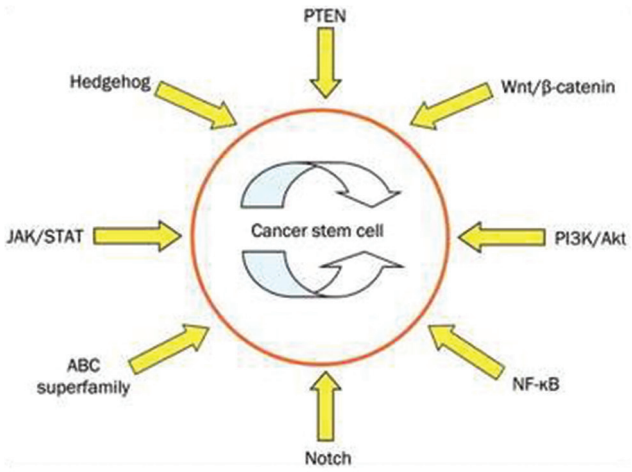

Figure 2: Signal pathways related to cancer stem cells

INVASION AND METASTASIS

CSCs have characteristic properties which are self-renewing ability to differentiate into diverse phenotype; ability to initiate tumors from minute numbers and high chemoresistance.[15] The hierarchical theory of tumor development states that there are two types of CSCs: One stationary and one movable. The stationary CSCs are responsible for resistance to chemotherapy and radiotherapy and are unable to spread. They serve to increase the tumor volume. In contrast, movable CSCs are capable of migrating and are responsible for metastasis locoregionally and/or remotely.[16] Understanding the cellular mechanisms of invasion and metastasis involved in oral cancer is critical for developing new diagnostics and therapeutic modalities. CSCs offer a unique mechanism for metastasis which results in tumor growth not only at the primary site, but also at the distant sites. CSCs behavior is orchestrated *in vivo* in tissue-specific, "niche" microenvironments which support stem cells maintenance and resistance to anoikis. The niche also serves as an anchoring site for stem cells. Thus, targeting the crosstalk between CSCs and other cells of their supportive niche may provide an effective way to abort the tumorigenic function of these cells.[17] Many key players in the stem cell niches have conserved functions in both normal and malignant tissues, and thus play key roles in tumorigenesis and metastasis. The EMT of cells with high tumorigenic potential plays an important role in the invasion of carcinoma cells leading to tumor dissemination.[17] There is a loss of expression of epithelial markers such as E-cadherin and acquisition of mesenchymal markers such as vimentin and snail seen in EMT, which is considered as a major mechanism of cancer metastasis.[18] Similarly, Bmi-1 is a transcriptional repressor, involved in EMT and metastasis in oral CSCs. The ALDH + HNSCC cells, while exhibiting stemness, also overexpress Bmi-1 and Snail in comparison to the ALDH negative cells. Clinically, coexpression of ALDH, Snail, and Bmi-1 predicted the worst prognosis in HNSCC patients.[19] *In vitro* and *in vivo* studies have found that oral cells with increased expression of CD44 have greater migration, invasion and metastatic potential as compared to cell showing low expression of CD44. This

Table 1: Molecular markers implicated in Head and neck SCC CSC detection

References	Stem cell markers	Cancer cell lines studied
Barth *et al.*, 2004	CD34, CD117 (receptor of stem cell factor)	Oral cavity, pharynx, and larynx
Kojc *et al.*, 2005	CD34, transforming growth factor beta (TGF beta 1)	Larynx
Tan *et al.*, 2006	Stem cell factor	Nasopharynx (plasma)
Prince *et al.*, 2007	CD44, Bmi-1	HNSCC generated in immunodeficient mouse
Zhou *et al.*, 2007	CD133	HNSCC cell lines (hep-2)
Pries *et al.*, 2008	CD44	Hypopharynx, larynx, and oropharynx
Chiou *et al.*, 2008	Oct-4, Nanog, and CD133	Oral squamous cell carcinoma stem like cells
Chen *et al.*, 2009	Bmi-11, Pakt (+) CD133, CD44	Tongue (SASVO3)
Chen *et al.*, 2009	ALDH1	Immunodeficient mouse model
Wu and Wu, 2009	CD133	HNSCC

TGF: Transforming growth factor, ALDH: Aldehyde dehydrogenase, HNSCC: Head and neck squamous cell carcinoma

suggests that CD44 also plays a crucial role in metastasis and disease progression. Similarly, ALDH1 was expressed in human esophageal squamous cell carcinoma (SCC) and this expression was significantly associated with lymph node metastasis and poor survival.[20]

THERUAPEUTICS

The standard oncology treatments have incomplete, and temporary effects that only shrink the tumor, and the tumor tends to relapse due to multiple resistant mechanisms existing in CSCs.[10] The resistance to chemotherapy and radiotherapy is considered to be a common cause of treatment failure in multiple malignancies.[8] The evidence has established that CSC populations are more resistant to conventional cancer therapies than non-CSC populations. Therefore, the elimination of CSCs is crucial in treating malignancies.[21]

Complete eradication of cancers may require the targeting and elimination of CSCs. This represents a challenge because many pathways are shared by CSCs and their normal counterparts such as those involved in self-renewal.[6]

Targeting the tumor microenvironment

The tumor microenvironment can create a niche to nurse and protect CSCs from drug-induced apoptosis. Tumor angiogenesis has also been reported to be related to CSC survival and drug resistance. The expression of vascular endothelial growth factor (VEGF) has been correlated with microvasculature formation and tumor growth. Targeting VEGF with bevacizumab leads to the normalization of tumor vasculature and resulting in disruption of the CSC niche. As stromal environment and CSC niche play a vital role in the behavior of cancer cells, targeting the stem cell niche directly can weaken the source of nutrition and change the essential signals needed by CSCs to proliferate.[22] Hypoxia also plays a key role in tumor progression and hypoxic tumor microenvironment in turn has a control over the CSCs. Hence, when the antiangiogenic agents are administered in combination with CSC targeted drugs, more effective results are attained in cancer therapy, along with inhibiting hypoxia inducible factors (HIF).[10] The therapeutic aspect should target hypoxia curbing these hypoxic niches by HIF-inhibitors, NFκBinhibitors, nutraceuticals, and antioxidant therapy. NF-κB degradation by inhibitor of κB (IκB) proteins may be a useful therapeutic solution under such circumstances. It has also been suggested that treatment with echinomycin blocks the hypoxia-induced stroma.[23]

Targeting drug-efflux pumps

The stem cells have an ability to pump drugs out of the cell with the help of the ABC family of drug transporters. If these efflux pumps could be inhibited, CSCs could be more susceptible to current or newly designed chemotherapeutic agents used.[9]

Targeting the molecular signaling pathways

The hedgehog signaling pathway

The Hh pathway is essential for the maintenance of stem cells. It also plays a crucial role in development and patterning during mammalian embryogenesis. Recent researchers have suggested that the Hh pathway is essential for the maintenance of CSCs in various human cancers including pancreatic cancer, colorectal cancer, and gastric cancer. It is also responsible for treatment resistance of cancer cell. Thus, inhibitors which obstruct any step of the Hh signaling pathway may result in depletion of CSCs and overcome the treatment resistance.[24]

The Notch signaling pathway

Notch signaling pathway plays crucial roles in cell-cell communication and in multiple cell fate decisions during embryonic development and adult life. Furthermore, Notch functions as an oncogenic protein in most human cancers including cervical, colon, lung, head and neck, prostate, pancreatic cancer, etc., while it may act as tumor suppressor in skin cancer, hepatocellular carcinoma, and SCLC. The Notch pathway is often over-activated in a variety of cancers, and it is believed that targeting Notch can help in the elimination of CSCs.[25]

Wnt signaling pathway

The canonical Wnt signaling pathway plays an important role in self-renewal and the maintenance of stem cells and CSC in soft tissues such as skin, intestine, and mammary gland. However inactivating, mutations of APC tumor suppressor or oncogenic mutations of b-catenin may result in the dysregulation of Wnt/β-catenin pathway in cancer cells or CSCs, which induces the neoplastic proliferation.[26] The Wnt pathway can be inhibited by Wnt inhibitory factors, Wnt antagonists, and condition al knockout of β-catenin. Extracellular molecules antagonize the Wnt signaling pathway by preventing ligand receptor interactions.

Targeting cancer stem cells markers

Targeting surface markers

CSCs in various tumors express specific surface markers, such as CD133 in hepatocellular and gastriccancer, CSCs 50, CD9, CD24, CD26, in human malignant mesothelioma CSCs, CD44, CD24, and ESA, in pancreatic CSCs. Among these markers, CD133 is considered the most important CSC associated marker identified so far. The evidence by Rappa *et al*. has suggested that CSCs expressing CD133 display strong resistance to chemotherapy and radiotherapy. They found that down regulation of CD133 using short hairpin RNAs in human metastatic melanoma lead to slower cell

growth, decreased ability to form spheroids, reduced cell motility, and reduced capacity to metastasize.

Targeting drug detoxifying enzyme

ALDH superfamily not only acts as a marker for both normal and CSCs but may also play important functional roles in self-protection, differentiation, and expansion. Thus, ALDH can act as a drug-detoxifying enzyme and be responsible for therapeutic resistance.

Induction of cancer stem cell differentiation

Apart from elimination therapies, another way to control the tumor progression is to induce differentiation of CSCs. This will force CSCs to differentiate terminally and lose their self-renewal property. Although many reagents have been studied in differentiation therapy, currently, only two kinds of anticancer drugs can affect cancer cell differentiation: Retinoic acids and drugs targeting tumor epigenetic change. The combined use of differentiation-inducing agents and chemotherapy represents an effective approach to eliminate CSCs.[10]

Role of salinomycin as a drug for targeting cancer stem cells

It was a great surprise when Gupta *et al*. showed in 2009 that salinomycin selectively kills human breast CSCs.[27] It was further demonstrated that salinomycin, in contrast to the anti-breast cancer drug paclitaxel, selectively reduces the proportion of CD44 high/CD24 low CSCs in cultures of mixed populations of HMLER-shEcad cells, and control cells that had not undergone EMT. According to the primary finding, salinomycin induces massive apoptosis in human cancer cells that display different mechanisms of drug and apoptosis resistance. Then, subsequent study demonstrated that salinomycin is able to overcome ABC transporter-mediated multidrug resistance and apoptosis resistance in the human acute myeloid leukemia stem cells.[28] Salinomycin has recently been shown to target CSCs in different types of human cancers, including gastric cancer, osteosarcoma, colorectal cancer, SCC, and prostate CSCs, suggesting that salinomycin may be effective in CSCs of many, if not all, types of human cancers.[29] There is a growing evidence that salinomycin targets CSCs as well as kills more differentiated non-CSC tumor cells and, most importantly, cancer cells that display efficient mechanisms of resistance to cytotoxic drugs, radiation, and apoptosis.

CONCLUSION

CSCs are responsible not only for tumor initiation, development, and metastasis but also for therapeutic resistance. Traditional approach can only shrink the tumors by killing the active tumor cells but miss the quiescent CSCs which are responsible for recurrence. Thus, new treatments targeting CSCs are necessary which will provide a novel and promising approaches for CSC targeted cancer therapy. The novel idea of targeting oral cancer relies around the encircled oral CSCs and its niche. However, CSCs and normal stem cells share many properties, thus, targeting CSCs may unfortunately affect normal stem cells too. Therefore, more precise therapies which can selectively target CSCs but spare normal stem cells are the need of the hour.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Zhang Z, Filho MS, Nör JE. The biology of head and neck cancer stem cells. Oral Oncol 2012;48:1-9.
2. Spillane JB, Henderson MA. Cancer stem cells: A review. ANZ J Surg 2007;77:464-8.
3. Sridharan G. The concept of cancer stem cell in oral squamous cell carcinoma. J Tumor 2014;2:257-60.
4. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, *et al*. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994;367:645-8.
5. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3:730-7.
6. Kaur S, Singh G, Kaur K. Cancer stem cells: An insight and future perspective. J Cancer Res Ther 2014;10:846-52.
7. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003;100:3983-8.
8. Chen K, Huang YH, Chen JL. Understanding and targeting cancer stem cells: Therapeutic implications and challenges. Acta Pharmacol Sin 2013;34:732-40.
9. Prince ME, Ailles LE. Cancer stem cells in head and neck squamous cell cancer. J Clin Oncol 2008;26:2871-5.
10. Rastogi P. Emergence of cancer stem cells in head and neck squamous cell carcinoma: A therapeutic insight with literature review. Dent Res J (Isfahan) 2012;9:239-44.
11. Lu H, Shi S, Gong T, Zhang Z, Sun X. Cancer stem cells: Therapeutic implications and perspectives in cancer therapy. Acta Pharm Sin B 2003;3:65-75.
12. Rampias T, Boutati E, Pectasides E, Sasaki C, Kountourakis P, Weinberger P, *et al*. Activation of Wnt signaling pathway by human papillomavirus E6 and E7 oncogenes in HPV16-positive oropharyngeal squamous carcinoma cells. Mol Cancer Res 2010;8:433-43.
13. Sinha N, Mukhopadhyay S, Das DN, Panda PK, Bhutia SK. Relevance of cancer initiating/stem cells in carcinogenesis and therapy resistance in oral cancer. Oral Oncol 2013;49:854-62.
14. Yu CC, Chang YC. Enhancement of cancer stem-like and epithelial mesenchymal transdifferentiation property in oral epithelial cells with long term nicotine exposure: Reversal by targeting SNAIL. Toxicol Appl Pharmacol 2013;266:459-69.

15. Rattan R, Ali Fehmi R, Munkarah A. Metformin: An emerging new therapeutic option for targeting cancer stem cells and metastasis. J Oncol 2012;2012:928127.
16. Allegra E, Trapasso S. Cancer stem cells in head and neck cancer. Onco Targets Ther 2012;5:375-83.
17. Papagerakis S, Pannone G, Zheng L, About I, Taqi N, Nguyen NP, *et al*. Oral epithelial stem cells - implications in normal development and cancer metastasis. Exp Cell Res 2014;325:111-29.
18. Kong D, Li Y, Wang Z, Sarkar FH. Cancer stem cells and epithelial-to-mesenchymal transition (EMT)-phenotypic cells: Are they cousins or twins? Cancers (Basel) 2011;3:716-29.
19. Yu CC, Lo WL, Chen YW, Huang PI, Hsu HS, Tseng LM, *et al*. Bmi-1 regulates snail expression and promotes metastasis ability in head and neck squamous cancer-Derived ALDH1 positive cells. J Oncol 2011;2011. pii: 609259.
20. Wang SJ, Wong G, de Heer AM, Xia W, Bourguignon LY. CD44 variant isoforms in head and neck squamous cell carcinoma progression. Laryngoscope 2009;119:1518-30.
21. Lacerda L, Pusztai L, Woodward WA. The role of tumor initiating cells in drug resistance of breast cancer: Implications for future therapeutic approaches. Drug Resist Updat 2010;13:99-108.
22. Routray S, Mohanty N. Cancer stem cells accountability in progression of head and neck squamous cell carcinoma: The most recent trends! Mol Biol Int 2014;2014:375325.
23. Martinez-Outschoorn UE, Trimmer C, Lin Z, Whitaker-Menezes D, Chiavarina B, Zhou J, *et al*. Autophagy in cancer associated fibroblasts promotes tumor cell survival: Role of hypoxia, HIF1 induction and NFκB activation in the tumor stromal microenvironment. Cell Cycle 2010;9:3515-33.
24. Chen YJ, Sims-Mourtada J, Izzo J, Chao KS. Targeting the hedgehog pathway to mitigate treatment resistance. Cell Cycle 2007;6:1826-30.
25. Qiao L, Wong BC. Role of Notch signaling in colorectal cancer. Carcinogenesis 2009;30:1979-86.
26. Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature 2005;434:843-50.
27. Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA, *et al*. Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell 2009;138:645-59.
28. Naujokat C, Steinhart R. Salinomycin as a drug for targeting human cancer stem cells. J Biomed Biotechnol 2012;2012:950658.
29. Fuchs D, Heinold A, Opelz G, Daniel V, Naujokat C. Salinomycin induces apoptosis and overcomes apoptosis resistance in human cancer cells. Biochem Biophys Res Commun 2009;390:743-9.