REVIEW ARTICLE

Cancer Stem Cells, the Cellular Signaling, and Potential Therapeutic Targets in Lung Cancer

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Abstract

Recent studies in the field of cancer cell biology have pointed out that the cancer cells having stem cell property in tumor may contribute aggressive phenotypes of cancers. A limited population of cancer cells exhibiting high ability to generate new tumor named "cancer stem cells (CSCs)" has garnered significant attention in these years and was linked with the initiation of primary tumor and cancer cells as well as the successful establishment of metastatic tumors. Based on the theory that stem cells preserve their ability to generate the new clones with different cell lineages because of their pluripotency, CSCs similarly exert its tumorigenicity. Besides, CSCs were shown to highly resist to currently used anticancer drugs and have augmented ability to metastasis. It is worthy highlighted that the CSCs that survive after chemotherapeutic treatment are believed to be the cause of disease relapse. Taken together, these evidences have supported the development of CSC-based targeted therapy. This review aimed at describing the fundamental information regarding the role of CSCs in lung cancer and its cellular signaling that may benefit the understanding and support the development of CSC-targeting research.

Keywords: Cancer stem cells, CSC-targeting therapy, cell signaling, drug target, lung cancer.

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Introduction

Studies have revealed that tumor contains several cancerous cells exhibiting heterogeneity. In termed of stem cell property, similar to the normal tissue, it is known that the stem cells are located at the top of tissue organization and these stem cells exhibiting self-renewal capacity and differentiation are responsible for the generation of cells in the tissue. Likewise, in tumor tissue, the cancer stem cells (CSCs) having self-renewal and differentiation abilities have been discovered and accepted as a dominant driver of cancer malignancy.

It was demonstrated that this unique cancer cell population possessing stem cell signals are important for cancer initiation, progression, and disease relapse.[1] In lung cancer, cancer stemness (CS) is described to be pivotal phenomena relating to cancer recurrence, drug resistance, and poor prognosis.[2] Several studies have pointed out that certain stem cell-associated signals including those that control cell survival and proliferation have across-talk effects with cellular stem cell functions. Interestingly, various cell signals were demonstrated to be essential for CSC maintenance; therefore, the identified signals controlling CSCs could be targets for CSC-targeting therapy. As CSCs and their underlying signals were believed to drive cancer aggressive behaviors, the use of small molecule inhibitors may attenuate the stemness in the tumor and benefit the clinical outcome.

Regarding CSC biology, it was known that survival pathways including c-Myc and Akt pathways play essential roles in controlling CSC behaviors, properties, and maintenance. Therefore, CSC-targeting therapy is highlighted as a novel cancer therapy. Since, Akt has been long implicated in several aspects facilitating tumor growth, survival and resistance in various types of cancer.[3,4] PI3K/Akt signaling pathway is important in prostate cancer stem-like cell maintenance and targeting PI3K was proposed to be helpful for treatment of prostate cancer via eliminating the prostate cancer stem-like cells.[5] The essential role of Akt signaling pathway in lung cancer was also demonstrated.[6] Role of c-Myc on CSCs has been intensively revealed. *Myc* is a family of regulator genes and proto-oncogenes which has been recognized as an essential regulator of CSCs.[7] A recent study revealed that overexpression of c-Myc resulted in an activation of numerous downstream genes, leading to conventional therapy-resistance in CSCs. c-Myc stability has been demonstrated to be a key factor determining its cellular activities and was shown to be regulated via PI3K/Akt pathway.[8,9] Swords et al.[10] demonstrated that when Akt pathway was disrupted, c-Myc was rapidly decreased as a result of destabilization and enhanced degradation. Besides, recent research has revealed the important impact of Akt/GSK3β pathway in regulation of c-Myc stability and ubiquitin-proteasomal degradation.[11] Akt signaling pathway not only regulates the stabilization of c-Myc but also affects the cellular function of this protein.[12]

Lung cancer

Lung cancer is a cancer that initiates in the lungs and is one of the leading causes of death worldwide.[13] Every year, over 1.8 million of lung cancer patients have been diagnosed.[14] It is caused by bronchial mucosa cells that have been irritated for a long time, so it may be called a bronchogenic carcinoma. In 2018, 2.09 million of

new cases and 1.76 million of deaths have been reported. The cancer incidence and death rates have increased considerably compared to 2012. Smoking is still the major risk factor of this cancer.[15] Most lung cancer patients have not shown any obvious symptoms in the initial stages. There are signs indicating the occurrence of the disease when there are more cancer growths, including chronic cough, chest pain, fatigue, shortness of breath, pain while breathing or coughing, and weight loss. Some of rare symptoms may occur such as wheezing, changes in fingertip and nail shape, high fever, difficult swallowing, hoarseness, and swelling of face and neck.[16]

There are various risk factors for lung cancer including air pollution, genetic factor, and tobacco smoking. These factors are the causes of cancer development.[16] Air pollution, both indoor and outdoor, acquires more than 30% of the causes of lung cancer.[17] The release of chemicals (SO_2, NOx, CO, and heavy metals) to the air and particulate matter (PM) are considered important causes of air pollution and directly affect the respiratory system.[18] In Taiwan, PM 2.5 was found to affect adenocarcinoma lung cancer incidence and the survival of patients.[19] Factors that cause cancer may come from external factors, but sometimes internal factors can also affect cancer risk such as genetic factors. If the family has a history of lung cancer, it will enhance the risk of disease as well.[16] Genetic variation in the 5p15.33 *TERT-CLPTM1L* region affects lung cancer risk in non-smokers.[20] Mutation of the epidermal growth factor receptor (EGFR) can induce cancer and the EGFR mutation in NSCLC patients is more common in female (over 40%) than male (less than 15%). Many research works have reported that tobacco smoking may be the major cause of this cancer, with the risks of 90% and 60% for male and female, respectively.[21] In the smoking patients, the predicted prognosis is poorer than the non-smoking ones.[22] Tobacco smoke contains many carcinogenic compounds (such as polyaromatic hydrocarbons, *N*-nitrosamines, acetaldehyde) that can cause lung cancer in smokers as well as secondhand smokers.[23]

There are 2 subtypes of lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), which accounts for approximately 15% and 85% of all lung cancers, respectively.[24] NSCLC, the main type of lung cancer, is categorized into 3 subtypes, including squamous cell lung carcinoma (25-30%) which has a strong relationship with smoking, large-cell lung carcinoma (5-10%) which starts in the middle of the lungs and can spread into nearby lymph nodes, and lung adeno-carcinoma (40%) which is a key type of lung cancer. Lung adenocarcinoma has a slow growth rate compared to other types of lung cancer and can be detected before it spreads outside the lungs.[25]

There are many options to cure lung cancer based on the cancer type, stage, and patient's condition. The first treatment is surgical treatment, which is suitable for patients with stage I and II lung cancer that does not spread to lymph nodes.[26] The second one is non-surgical treatment such as targeted therapy, chemotherapy, and immunotherapy. For NSCLC, the driver mutations are an important part of NSCLC diagnosis. A previous study showed that 50% of NSCLC patients had oncogenic drivers, and targeted therapy could improve their overall survival. This is the reason why FDA approved tyrosine kinase inhibitors (TKI), one type of targeted therapeutic drugs, for NSCLC treatment. However, the targeted therapy of SCLC has not yet been approved by the FDA. For immunotherapy, immune checkpoint inhibitors (ICIs) can be used in combination with chemotherapy.[24] The last treatment is radiotherapy

which is very essential in lung cancer treatment and is used in palliative care. It can improve therapeutic response via a combination of immunotherapeutic agents and radiation.[27]

CSCs and their role in lung cancer

Stem cells are specialized cells that have the ability to self-renew and differentiate into several different types of cells. Even with a small population, stem cells are essential for replenishing aging cells and repairing damaged tissues.[28] CSCs are a small population inside the tumors holding stemness characters which promote tumor initiation, tumor cell metastasis and tumor recurrence. Currently, CSCs are a key factor contributing to low rate of successful treatments.[29] Available chemotherapeutic drugs induce programmed cell death in normal cancer cells but not in CSCs.[30] Other than that, CSCs show several characteristics like normal pluripotent stem cells including self-renewal, specific gene expression and protein markers, and the signaling pathways.[31] These characteristics point out that CSCs are associated with tumor development and progression. In addition, previous research reported that the intrinsic and the extrinsic alterations in the tumor microenvironment, together with the mutations and the epigenetic regulations, are involved and responsible for CSC development.[32] CSCs are a small sub-population of cells within tumors that possess the same characteristics as normal stem cells (NSCs). CSCs have the ability to initiate tumor formation, widespread proliferation, and become resistant to cancer chemotherapeutic drugs.[31] The overall contributions of CSCs on chemotherapeutic drug resistance and cancer relapse are concluded in Figure 1.[33]

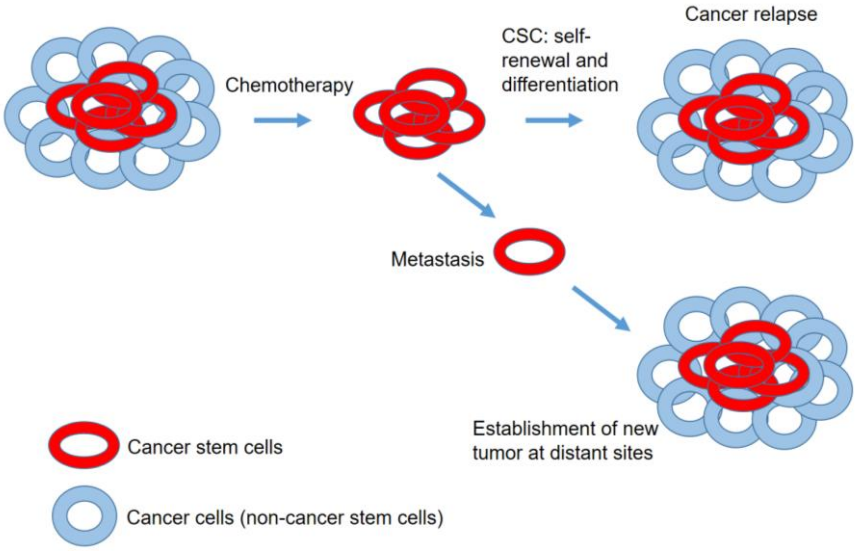

Figure 1. CSC biology and contribution to metastasis and disease relapse. CSCs (red) can self-renew and are developed in cancer to form tumor bulk (blue). When the tumor grows, cells have a limited capacity for growth or all cells may form disseminated malignancies. Therefore, cells are resistant to drugs, leading to cancer recurrence.

In 2012 in the United States, there were 226,160 patients diagnosed with lung cancer and 160,340 deaths were reported.[34] Although the diagnosis and treatment of lung cancer have been advanced over the past decade, the prognosis remains poor due to its resistance to therapy, rapid tumor growth, and its ability to spread.[35] CSCs are responsible for the aggressive phenotypes of lung carcinoma. CSCs express stem cell markers in lung cancer including CD133, CD44, ALDH, Oct4, and Nanog.[36] Moreover, it is important to be able to distinguish between NSCLC and SCLC on the basis of CSC characteristics, which is responsible for treatment strategies and prognosis.[37]

CSC markers

CSCs are a potential driving force of initiating new tumors and progression of cancer because of the self-renewal, drug resistance, metastasis, and cancer recurrence.[38] Up-regulation of specific stem cell protein markers including CD133 and ALDH1A1 are related to tumorigenesis and chemoresistance properties of CSCs.[39]

CD133 CD133 is a common marker of lung CSCs.[40] It is a cell surface glycoprotein that consists of 5 transmembrane domains and 2 large glycosylated extracellular loops.[41] The findings from previous research indicated that $CD133^+$ cells had significantly higher abilities for self-renewal, cancer initiation, and chemoresistance features when compared to $CD133^-$ cells.[42] Moreover, previous research also found $CD133^+$ cells with CSC character in SCLC cell lines. Therefore, CD133 can be represented as a major lung CSC marker.[43] High expression of CD133 protein is linked to poor prognosis in NSCLC patients, since the expression of CD133 is correlated with higher cancer stage in lung cancer.[40]

Aldehyde dehydrogenase (ALDH) Another marker used to identify and isolate the CSCs is ALDH. ALDH is an intracellular enzyme that regulates the cell differentiation of NSCs.[44] It is involved in detoxification process and drug resistance in stem cells.[45] One of the members of the ALDH family is ALDH1, a cytosolic isoenzyme. The expression of ALDH1 in lung cancer cells indicated highly tumorigenic and cancer cell cloning properties.[46] Moreover, expression of ALDH1A1 in CSCs displayed chemotherapy and epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) resistance.[47]

Transcription factors

Increases in expressions of transcription factors such as Nanog, Sox2, and Oct4 were observed in CSCs. These transcription factors play a key role in pluripotency and self-renewal activities.The overexpressions of these markers that affects proliferation property and self-renewal were observed in CSCs rather than in embryonic stem cells (ESCs) or normal cells.[48] Transcription factors of stemness can modulated the stem phenotype such as self-renewal and pluripotency in NSCs and CSCs. Sox2, Nanog and Oct4 are commonly transcriptional factors that mediate CSC properties in various cancers, including lung cancer.[49,50] Oct4/Sox2/Nanog complex recruits important transcriptional factors to induce stemness-regulating proteins.[51] It was demonstrated that epidermal growth factor receptor (EGFR), an important growth factor receptor dominantly found in the lungs and other aggressive cancers, plays a critical role in initiation and maintaining the cellular signals, leading to the inductions of stem cell transcription factors (Figure 2).[52]

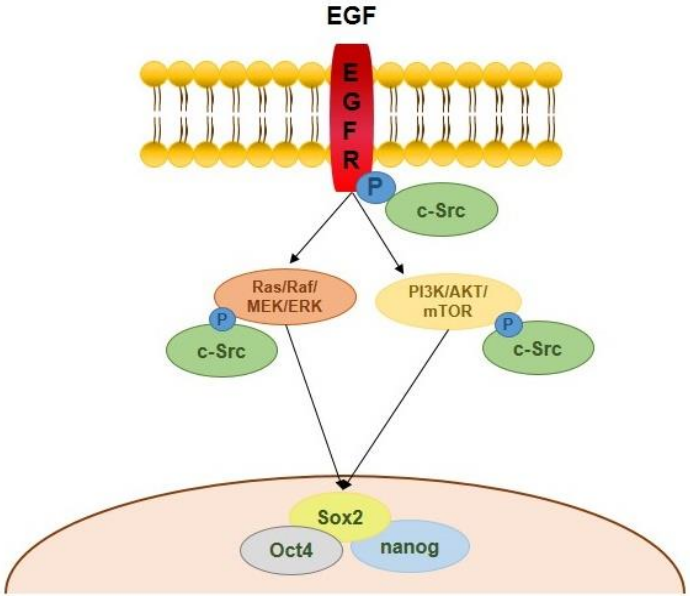

Figure 2. Role of EGF in regulating survival molecules on stemness transcriptional factor complex.

Nanog Nanog is a critical transcription factor in maintaining the capacity of self-renewal of ESCs in the development of embryo.[53] In lung cancer patients with overexpression of this protein, the prognosis is worsened. Therefore, Nanog protein is used as a key predictor in the lung cancer prognosis.[54] Nanog can control the self-renewal and cell potency of ESCs. In addition, the combination of Nanog with other proteins including Oct4, Sox2 and Lin28 may be used to induce effective reprogramming process in fibroblasts to provide the induced pluripotent stem cells (iPSCs) (Figure 3).[55] Nanog does not only regulate self-renewal and induce pluripotent stem cells but also displays a role in the regulation of ESC cell cycle. Previous research showed that overexpression of Nanog protein expedited S-phase entry of ESC clones. In physiological conditions, the C-terminal of Nanog bound to CDK6 and CDC25A at regulatory region, which mediated S-phase entry.[56] Moreover, NanogP8 was more expressed in T-cell acute lymphoblastic leukemia and the knockdown of NanogP8 could suppress proliferation of cells and self-renewal, and induce apoptosis and cell cycle arrest via p53 pathway.[57] In addition, downregulation of Nanog caused G0/G1 cell cycle arrest and reduced cyclin E expression and STAT3 in breast cancer cell line.[58] Nanog is also important in controlling ESC cell cycle. Therefore, the investigation of Nanog expression in lung cancer stem cells may be useful for the development of new therapies.

Sox2 Sox2 (sex determining region Y-box 2) is a stem cell transcription factor. Up-regulation of Sox2 is currently reported in various types of lung carcinoma such as SCLC, squamous cell lung carcinoma and lung adenocarcinoma.[52] Spheroid formation and CSC apoptosis are regulated by Sox2 protein level.[59] Previous research found that Sox2 plays a role in the repair the pluripotent of iPSCs and ESCs. In addition, Sox2 has been accepted as a powerful oncogene in numerous cancers, where it controls CSCs and is functionally related to hallmarks of cancer.[60]

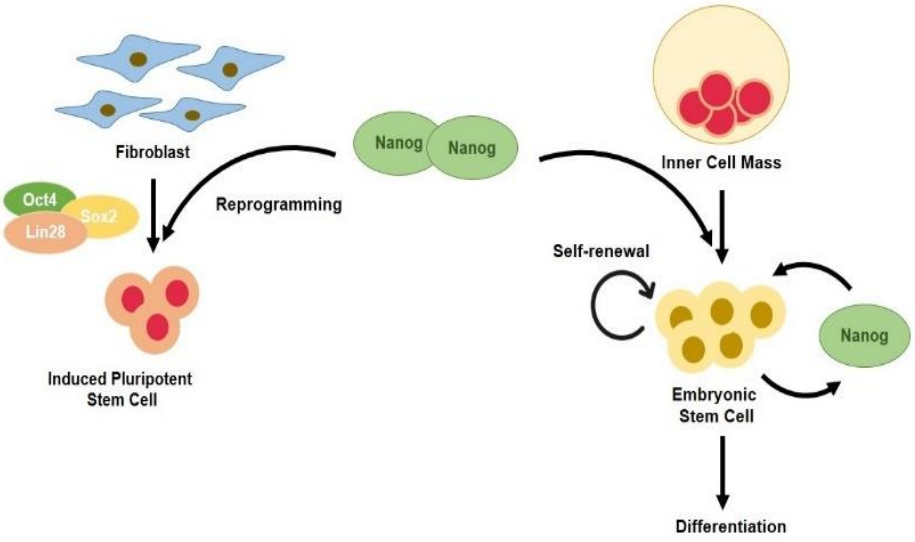

Figure 3. Role of Nanog in controlling self-renewal and pluripotency of stem cells.

Oct4 Oct4 (octamer-binding transcription factor 4) is one of the CSC transcription factors. It is an essential regulator of ESC fate. Cancer cells are similar in appearance to early embryonic cells, with common characteristics including deathless, undifferentiated, and invasive.[61] Dysregulation of overexpressed proteins during the embryonic stage can cause development of cancer. Oct4 is specifically expressed in the ESCs and a large amount is needed to maintain self-renewal of ESCs. This means that Oct4 is a main regulator of pluripotency in mammalian development.[62] A previous study has shown that the expression of Oct4 was a critical factor in keeping CSC characters in lung cancer with $CD133^+$ cells and suppression on Oct4 expression inhibited stemness phenotypes and metastasis feature in CSCs of lung cancer cells.[42]

CSC signaling pathways

Current research reported that CSCs have various similar characters to NSCs including self-renewal and differentiation. They also share many major pathways to remain their survivals.[63] CSCs have been reported to show various properties of embryonic or stem cell tissue and cancer development pathways including Wnt, Hedgehog, and Notch which have been well-maintained as self-renewal property of stem cell.[64] So, activation of these signaling pathways may give a significant role in the extension of CSCs and therefore resistant to drug.[65]

Wnt signaling pathway Wnt signaling is one of the key regulators of tissue morphogenesis throughout embryogenesis, with the support of 19 Wnt proteins, 2 co-receptors, 10 Frizzled (Fz) receptors, and numerous non-Fz receptors. In addition, Wnt pathway regulates a wide range of functions that include stem cell survival and maintenance stem cell properties. To activate the Wnt/β-catenin pathway, a Wnt ligand binds to the receptor (Fz receptor) on the cell surface and LRP5/6 co-receptor and further forms Wnt-Fz-LRP6 complex. Fz can recruit Dvl and cause the phosphorylation of LRP5/6 and Axin recruitment. The interaction of Wnt and Fz receptor results in the arrest of Axin-mediated phosphorylation of β-catenin and causes

translocation of β-catenin into the nucleus to create complexes with TCF/LEF which further activates the target genes.[66]

Previous studies have reported that the Wnt signaling has more relationship with stem cells.[67] Previous evidence suggested that the activation of Wnt/β-catenin signaling increased the drug resistance to IFN-α/5-FU combination therapy.[68] Furthermore, the translocation of the β-catenin into the nucleus induces the activation of target genes in cancer such as *MYC* (Figure 4).[69]

Canonical Notch signaling Notch signaling is an essential pathway in developmental programs and differentiation.[70] Notch signaling can be activated when ligand binding occurs, resulting in release of Notch intracellular domain (NICD) via the function of two proteolytic cleavages (ADAM and γ-secretase complex). NICD is transferred into the nucleus and then binds to CSL transcription factor, leading to activation of various downstream target genes of Myc, Nanog, Oct4, and Sox2. These are pertinent to self-renewal (Figure 5).[71,72]

Hedgehog pathway The Hedgehog (Hh) signaling pathway is an important pathway for various organ development during embryogenesis such as lungs, heart, and gastrointestinal tract, via the control of proliferation and differentiation.[73,74] The essential proteins in the Hh signaling pathway include Hh ligands, a transmembrane protein Smoothened (Smo), and Glis1-3 transcription factors that regulate the activation or suppression of the pathway.[75]

For the activation of Hh pathway, three mammalian counterparts, sonic hedgehog (Shh), Indian hedgehog (Ihh) and desert hedgehog (Dhh) bind to Patched 1 (Ptch1) and allow it to leave from the cell surface. Then, Smo moves into the cilium and activate Gli transcription factors. In this system, Gli activator forms enter the nucleus and induce the Hh target genes transcription. In addition, when ligands are absent, Ptch1 is still located in the cilium and blocks Smo entry. Gli transcription factors exist in repressor forms that interrupt transcription process of target genes.[73]

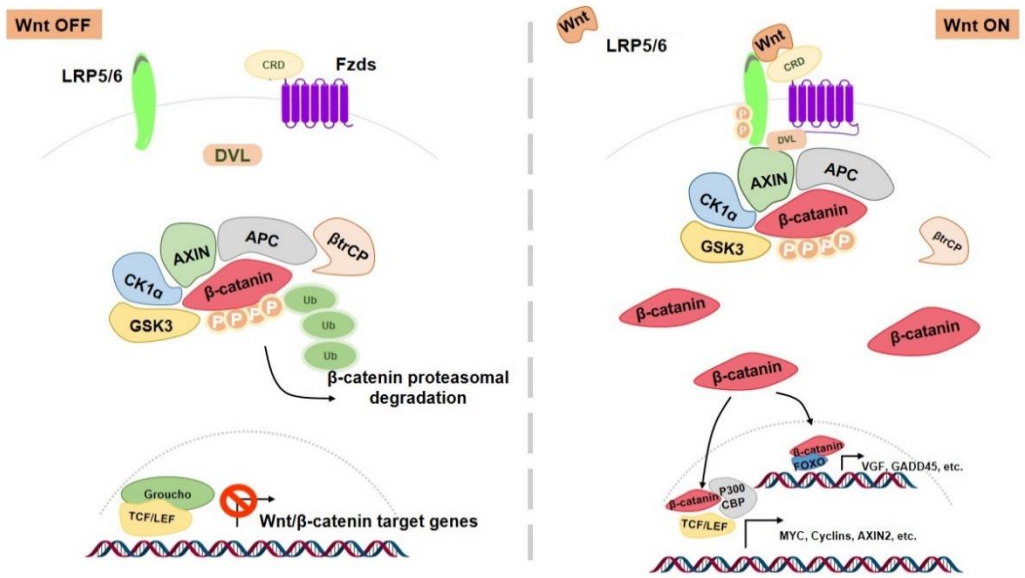

Figure 4. Wnt/β-catenin signaling cascade.

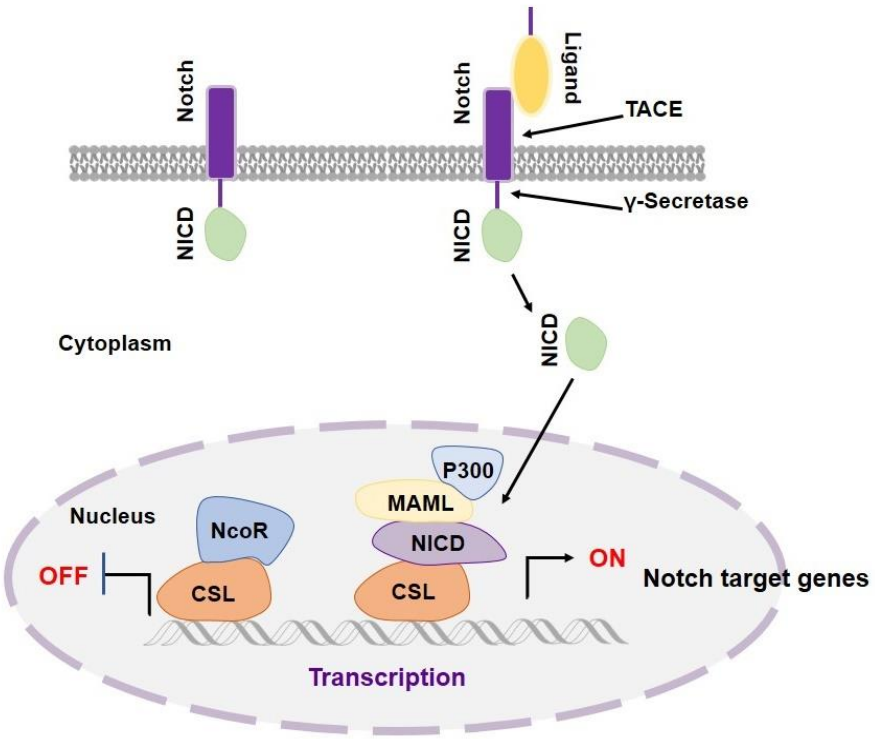

Figure 5. Schematic of Notch signaling.

Active Hh signaling can potentially cause cancer treatment failure by reducing chemotherapeutic responses or actively creating more aggressive and therapy-resistant tumors. Previous research reported that treatment of glioma CSCs with an Hh inhibitor led to the reduction of cell proliferation, cell existence, and self-renewal, along with the decrease in the expression level of the stem cell genes including Sox2, Nanaog, and Oct4.[76]

c-Myc regulates CSC properties

c-Myc is the target gene in Wnt pathway. It plays a critical role in an oncogenic switch. For example, after suppression of β-catenin/TCF-4 activity in colorectal cancer cells, the decrease in *c-Myc* gene expression led to the transcription of *p21*, resulting in differentiation and triggering G1 arrest.[77]

Notch signaling can activate PI3K/Akt signaling pathway[78] and c-Myc transcription. A recent research reported that in CRC cell lines, the activation of the Notch pathway by mastermind-like protein (MAML)-1 could transcriptionally bind to c-Myc and cyclin D1.[79] Myc and cyclin D1 are related to the progression of the cell cycle. The suppression of Notch pathway may involve in inhibition of the cell cycle progression, causing anticancer effect.[80-82]

Moreover, self-renewal in colorectal CSCs is dependent on the Hedgehog-GLI via connection with the Wnt pathway.[83] GLI-1 can reduce the expression of c-Myc and suppress the cell proliferation.[84] It indicates that c-Myc transcription factor is important in CRC therapy.

c-Myc maintains the chemoresistance

c-Myc protein has a significant role in drug resistance. The overexpression of c-Myc triggers downstream genes which are related to cell cycle regulation and genomic instability.[85-87] Several chromosomal abnormalities occur when c-Myc induces genomic instability such as elevation of elements, centromere and telomere fusions, DNA double strand breaks, and genetic mutation.[87-90] In addition, the aggregation of genomic instability increases the susceptibility of tumor cells to DNA-damaging agents.[91,92]

Akt/c-Myc signaling pathway in CSCs

PI3K/Akt signaling pathway is important to cancer cell differentiation, cell proliferation, and cell survival under both physiological and pathological conditions.[93] Previous research showed that activation of the PI3K/Akt signaling pathway led to epithelial mesenchymal transition (EMT) and enhanced CSC phenotypes involved in prostate cancer radioresistance.[94] PI3K/Akt pathway is significant for prostate CSC maintenance and that targeting PI3K signaling may be beneficial in prostate cancer treatment by eliminating prostate CSCs.[5] *Myc* is characterized as a regulator genes and proto-oncogenes which encode important nuclear transcription factors. As c-Myc has been recognized as an essential regulator of CSCs.[7] Swords et al. demonstrated that when Akt pathway was disrupted, c-Myc was found to be rapidly decreased as a result of destabilization and enhanced degradation.[10] Not only stability of the protein requires Akt/mTOR activity, but c-Myc cellular function was shown to depend on Akt activity.[12] A previous study indicated that threonine 308 (Thr308) and serine 473 (Ser473) were the two main sites of Akt phosphorylation. The activation of Thr308 phosphorylation may increase the Akt enzymatic activity.[95] Recent research have revealed the important impact of Akt/GSK3β pathway in regulation of c-Myc stability and ubiquitin-proteasomal degradation. The phosphorylation at Threonine 58 (Thr58) and serine 62 (Ser62) of c-Myc is significant for c-Myc ubiquitin-proteasomal degradation.[11]

Conclusion

CSCs among cancer cell population drive carcinogenesis, disease progression, metastasis, and drug resistance. Importantly, the survived subpopulation possessing CSC characteristics after cancer treatment renders disease relapse. In order to completely control cancer, it is quite essential to understand the basis mechanisms regulating and maintaining CSCs. Several survival and stem cell regulatory pathways have been identified to be critical factors for CSC function and maintenance and these cell signals were co-related with the prognosis of cancers. Inhibition of CSCs, whether it be alteration of CSC markers or inhibition of CSC signaling pathways, may be a possible alternative for lung cancer treatment under such circumstances.

References

1. Huang T, Song X, Xu D, Tiek D, Goenka A, Wu B, et al. Stem cell programs in cancer initiation, progression, and therapy resistance. Theranostics. 2020;10(19): 8721-43.

2. Sowa T, Menju T, Sonobe M, Nakanishi T, Shikuma K, Imamura N, et al. Association between epithelial-mesenchymal transition and cancer stemness and their effect on the prognosis of lung adenocarcinoma. Cancer med. 2015 Dec;4(12):1853-62.
3. Zhang HF, Wu C, Alshareef A, Gupta N, Zhao Q, Xu XE, et al. The PI3K/AKT/c-MYC axis promotes the acquisition of cancer stem-like features in esophageal squamous cell carcinoma. Stem Cells. 2016 Aug;34(8):2040-51.
4. Xia P, Xu XY. PI3K/Akt/mTOR signaling pathway in cancer stem cells: from basic research to clinical application. Am J Cancer Res. 2015 Apr 15;5(5):1602-9.
5. Dubrovska A, Kim S, Salamone RJ, Walker JR, Maira SM, García-Echeverría C, et al. The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. Proc Natl Acad Sci USA. 2009 Jan 6; 106(1):268-73.
6. Srinual S, Chanvorachote P, Pongrakhananon V. Suppression of cancer stem-like phenotypes in NCI-H460 lung cancer cells by vanillin through an Akt-dependent pathway. Int J Oncol. 2017 Apr;50(4):1341-51.
7. Murphy MJ, Wilson A, Trumpp A. More than just proliferation: Myc function in stem cells. Trends Cell Biol. 2005 Mar;15(3):128-37.
8. Elbadawy M, Usui T, Yamawaki H, Sasaki K. Emerging roles of c-Myc in cancer stem cell-related signaling and resistance to cancer chemotherapy: a potential therapeutic target against colorectal cancer. Int J Mol. Sci. 2019 May 11; 20(9):2340.
9. Deb TB, Coticchia CM, Dickson RB. Calmodulin-mediated activation of Akt regulates survival of c-Myc-overexpressing mouse mammary carcinoma cells. J Biol Chem. 2004 Sep 10;279(37):38903-11.
10. Swords RT, Schenk T, Stengel S, Gil VS, Petrie KR, Perez A, et al. Inhibition of the PI3K/AKT/mTOR pathway leads to down-regulation of c-Myc and overcomes resistance to ATRA in acute myeloid leukemia. Blood. 2015 Dec 3;126(23):1363.
11. Chanvorachote P, Sriratanasak N, Nonpanya N. C-myc contributes to malignancy of lung cancer: a potential anticancer drug target. Anticancer Res. 2020 Feb; 40(2):609-18.
12. Zhu J, Blenis J, Yuan J. Activation of PI3K/Akt and MAPK pathways regulates Myc-mediated transcription by phosphorylating and promoting the degradation of Mad1. Proc Natl Acad Sci USA. 2008 May 6;105(18):6584-9.
13. Sarode P, Mansouri S, Karger A, Schaefer MB, Grimminger F, Seeger W, et al. Epithelial cell plasticity defines heterogeneity in lung cancer. Cell Signal. 2020 Jan;65:109463.
14. Sears CR, Mazzone PJ. Biomarkers in lung cancer. Clin Chest Med. 2020 Mar; 41(1):115-27.
15. Bade BC, Cruz CSD. Lung cancer 2020: epidemiology, etiology, and prevention. Clin Chest Med. 2020 Mar;41(1):1-24.
16. Ahmad AS, Mayya AM. A new tool to predict lung cancer based on risk factors. Heliyon. 2020 Feb26;6(2):e03402.

17. Ning J, Li P, Zhang B, Han B, Su X, Wang Q, et al. miRNAs deregulation in serum of mice is associated with lung cancer related pathway deregulation induced by PM2.5. Environ Pollut. 2019 Nov;254(Pt A):112875.
18. Shahadin MS, Mutalib NSA, Latif MT, Greene CM, Hassan T. Challenges and future direction of molecular research in air pollution-related lung cancers. Lung Cancer. 2018 Apr;118:69-75.
19. Tseng CH, Tsuang BJ, Chiang CJ, Ku KC, Tseng JS, Yang TY, et al. The relationship between air pollution and lung cancer in nonsmokers in Taiwan. J Thorac Oncol. 2019 May;14(5):784-92.
20. Wang Y, Broderick P, Matakidou A, Eisen T, Houlston RS. Role of 5p15.33 (*TERT-CLPTM1L*), 6p21.33 and 15q25.1 (*CHRNA5-CHRNA3*) variation and lung cancer risk in never-smokers. Carcinogenesis. 2010 Feb;31(2):234-8.
21. Jin K, Wu M, Zhou JY, Yang J, Han RQ, Jin ZY, et al. Tobacco smoking modifies the association between hormonal factors and lung cancer occurrence among post-menopausal chinese women. Transl Oncol. 2019 Jun;12(6):819-27.
22. Hanagiri T, Sugio K, Mizukami M, Ichiki Y, Sugaya M, Yasuda M, et al. Significance of smoking as a postoperative prognostic factor in patients with non-small cell lung cancer. J Thorac Oncol. 2008 Oct;3(10):1127-32.
23. Steliga MA, Dresler CM. Epidemiology of lung cancer: smoking, secondhand smoke, and genetics. Surg Oncol Clin N Am. 2011 Oct;20(4):605-18.
24. Salehi-Rad R, Li R, Paul MK, Dubinett SM, Liu B. The biology of lung cancer: development of more effective methods for prevention, diagnosis, and treatment. Clin Chest Med. 2020 Mar;41(1):25-38.
25. Zappa C, Mousa SA. Non-small cell lung cancer: current treatment and future advances. Transl Lung Cancer Res. 2016 Jun;5(3):288-300.
26. Ginsberg RJ, Rubinstein LV, Group LCS. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Ann Thorac Surg. 1995 Sep;60(3):615-22.
27. Benveniste MF, Welsh J, Viswanathan C, Shroff GS, Cuellar SLB, Carter BW, et al. Lung cancer: posttreatment imaging: radiation therapy and imaging findings. Radiol Clin North Am. 2018 May;56(3):471-83.
28. Codony-Servat J, Verlicchi A, Rosell R. Cancer stem cells in small cell lung cancer. Transl Lung Cancer Res. 2016 Feb;5(1):16-25.
29. Eide CA, Druker BJ. Understanding cancer from the stem cells up. Nat Med. 2017 Jun 6;23(6):656-7.
30. Yang L, Shi P, Zhao G, Xu J, Peng W, Zhang J, et al. Targeting cancer stem cell pathways for cancer therapy. Signal Transduct Target Ther. 2020 Feb 7;5(1):8.
31. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001 Nov 1;414(6859):105-11.
32. Bao B, Ahmad A, Li Y, Azmi AS, Ali S, Banerjee S, et al. Targeting CSCs within the tumor microenvironment for cancer therapy: a potential role of mesenchymal stem cells. Expert Opin Ther Targets. 2012 Oct;16(10):1041-54.

33. Zakaria N, Satar NA, Abu Halim NH, Ngalim SH, Yusoff NM, Lin J, et al. Targeting lung cancer stem cells: research and clinical impacts. Front Oncol. 2017 May 5;7:80.
34. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012 Jan-Feb;62(1):10-29.
35. Wu X, Chen H, Wang X. Can lung cancer stem cells be targeted for therapies? Cancer Treat Rev. 2012 Oct;38(6):580-8.
36. Eramo A, Haas T, De Maria R. Lung cancer stem cells: tools and targets to fight lung cancer. Oncogene. 2010 Aug 19;29(33):4625-35.
37. Kitamura H, Okudela K, Yazawa T, Sato H, Shimoyamada H. Cancer stem cell: implications in cancer biology and therapy with special reference to lung cancer. Lung Cancer. 2009 Dec;66(3):275-81.
38. Sullivan JP, Minna JD, Shay JW. Evidence for self-renewing lung cancer stem cells and their implications in tumor initiation, progression, and targeted therapy. Cancer Metastasis Rev. 2010 Mar;29(1):61-72.
39. Suresh R, Ali S, Ahmad A, Philip PA, Sarkar FH. The role of cancer stem cells in recurrent and drug-resistant lung cancer. Adv Exp Med Biol. 2016;890:57-74.
40. Mizugaki H, Sakakibara-Konishi J, Kikuchi J, Moriya J, Hatanaka KC, Kikuchi E, et al. CD133 expression: a potential prognostic marker for non-small cell lung cancers. Int J Clin Oncol. 2014 Apr;19(2):254-9.
41. Hu Y, Fu L. Targeting cancer stem cells: a new therapy to cure cancer patients. Am J Cancer Res. 2012;2(3):340.
42. Chen YC, Hsu HS, Chen YW, Tsai TH, How CK, Wang CY, et al. Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. PloS one. 2008 Jul 9;3(7):e2637.
43. Singh S, Chellappan S. Lung cancer stem cells: molecular features and therapeutic targets. Mol Aspects Med. 2014 Oct;39:50-60.
44. Russo JE, Hilton J. Characterization of cytosolic aldehyde dehydrogenase from cyclophosphamide resistant L1210 cells. Cancer Res. 1988 Jun 1;48(11):2963-8.
45. Moreb J, Schweder M, Suresh A, Zucali JR. Overexpression of the human aldehyde dehydrogenase class I results in increased resistance to 4-hydroperoxy-cyclophosphamide. Cancer Gene Ther. 1996 Jan-Feb;3(1):24.
46. Sullivan JP, Spinola M, Dodge M, Raso MG, Behrens C, Gao B, et al. Aldehyde dehydrogenase activity selects for lung adenocarcinoma stem cells dependent on notch signaling. Cancer Res. 2010 Dec 1;70(23):9937-48.
47. Huang CP, Tsai MF, Chang TH, Tang WC, Chen SY, Lai HH, et al. ALDH-positive lung cancer stem cells confer resistance to epidermal growth factor receptor tyrosine kinase inhibitors. Cancer Lett. 2013 Jan 1;328(1):144-51.
48. Liu A, Yu X, Liu S. Pluripotency transcription factors and cancer stem cells: small genes make a big difference. Chin J Cancer. 2013 Sep;32(9):483-7.
49. Wang ML, Chiou SH, Wu CW. Targeting cancer stem cells: emerging role of Nanog transcription factor. Onco Targets Ther. 2013 Sep 4;6:1207-20.

50. Chiou SH, Wang ML, Chou YT, Chen CJ, Hong CF, Hsieh WJ, et al. Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell–like properties and epithelial–mesenchymal transdifferentiation. Cancer Res. 2010 Dec 15;70(24):10433-44.
51. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell. 2005 Sep 23;122(6):947-56.
52. Karachaliou N, Rosell R, Viteri S. The role of SOX2 in small cell lung cancer, lung adenocarcinoma and squamous cell carcinoma of the lung. Transl Lung Cancer Res. 2013 Jun;2(3):172-9.
53. Pan G, Thomson JA. Nanog and transcriptional networks in embryonic stem cell pluripotency. Cell Res. 2007 Jan;17(1):42-9.
54. Du Y, Ma C, Wang Z, Liu Z, Liu H, Wang T. Nanog, a novel prognostic marker for lung cancer. Surg Oncol. 2013 Dec;22(4):224-9.
55. Zhang W, Sui Y, Ni J, Yang T. Insights into the Nanog gene: A propeller for stemness in primitive stem cells. Int J Biol Sci. 2016 Oct 25;12(11):1372-81.
56. Zhang X, Neganova I, Przyborski S, Yang C, Cooke M, Atkinson SP, et al. A role for NANOG in G1 to S transition in human embryonic stem cells through direct binding of CDK6 and CDC25A. J Cell Biol. 2009 Jan 12;184(1):67-82.
57. Cao J, Li L, Chen C, Lv C, Meng F, Zeng L, et al. RNA interference-mediated silencing of NANOG leads to reduced proliferation and self-renewal, cell cycle arrest and apoptosis in T-cell acute lymphoblastic leukemia cells via the p53 signaling pathway. Leuk Res. 2013 Sep;37(9):1170-7.
58. Han J, Zhang F, Yu M, Zhao P, Ji W, Zhang H, et al. RNA interference-mediated silencing of NANOG reduces cell proliferation and induces G0/G1 cell cycle arrest in breast cancer cells. Cancer Lett. 2012 Aug 1;321(1):80-8.
59. Tian T, Zhang Y, Wang S, Zhou J, Xu S. Sox2 enhances the tumorigenicity and chemoresistance of cancer stem-like cells derived from gastric cancer. J Biomed Res. 2012 Sep;26(5):336-45.
60. Schaefer T, Lengerke C. SOX2 protein biochemistry in stemness, reprogramming, and cancer: the PI3K/AKT/SOX2 axis and beyond. Oncogene. 2020 Jan;39(2): 278-92.
61. Chang CC, Shieh GS, Wu P, Lin CC, Shiau AL, Wu CL. Oct-3/4 expression reflects tumor progression and regulates motility of bladder cancer cells. Cancer Res. 2008 Aug 1;68(15):6281-91.
62. Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, et al. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell. 1998 Oct 30;95(3):379-91.
63. Phi LTH, Sari IN, Yang YG, Lee SH, Jun N, Kim KS, et al. Cancer stem cells (CSCs) in drug resistance and their therapeutic implications in cancer treatment. Stem Cells Int. 2018 Feb 28;2018:5416923.
64. Takebe N, Miele L, Harris PJ, Jeong W, Bando H, Kahn M, et al. Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update. Nat Rev Clin Oncol. 2015 Aug;12(8):445-64.

65. Kurth I, Peitzsch C, Baumann M, Dubrovska A. The role of cancer stem cells in tumor radioresistance. In: Rajasekhar VK, editor. Cancer stem cells. Hoboken: John Wiley & Sons; 2014. p. 473-91.
66. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. Dev Cell. 2009 Jul;17(1):9-26.
67. Kühl SJ, Kühl M. On the role of Wnt/β-catenin signaling in stem cells. Biochim Biophys Acta 2013 Feb;1830(2):2297-306.
68. Noda T, Nagano H, Takemasa I, Yoshioka S, Murakami M, Wada H, et al. Activation of Wnt/β-catenin signalling pathway induces chemoresistance to interferon-α/5-fluorouracil combination therapy for hepatocellular carcinoma. Br J Cancer. 2009 May 19;100(10):1647-58.
69. Zhong Z, Virshup DM. Wnt signaling and drug resistance in cancer. Mol Pharmacol. 2020 Feb;97(2):72-89.
70. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science. 1999 Apr 30;284(5415):770-6.
71. Wang Z, Ahmad A, Li Y, Azmi AS, Miele L, Sarkar FH. Targeting notch to eradicate pancreatic cancer stem cells for cancer therapy. Anticancer Res. 2011 Apr;31(4):1105-13.
72. Koury J, Zhong L, Hao J. Targeting signaling pathways in cancer stem cells for cancer treatment. Stem Cells Int. 2017;2017:2925869.
73. Merchant AA, Matsui W. Targeting Hedgehog—a cancer stem cell pathway. Clin Cancer Res. 2010 Jun 15;16(12):3130-40.
74. Petrova R, Joyner AL. Roles for Hedgehog signaling in adult organ homeostasis and repair. Development. 2014 Sep;141(18):3445-57.
75. Matsui WH. Cancer stem cell signaling pathways. Medicine (Baltimore). 2016 Sep;95(1 Suppl 1):S8-S19.
76. Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. Curr Biol. 2007 Jan 23;17(2):165-72.
77. Van De Wetering M, Sancho E, Verweij C, De Lau W, Oving I, Hurlstone A, et al. The β-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell. 2002 Oct 18;111(2):241-50.
78. Parsons DW, Wang TL, Samuels Y, Bardelli A, Cummins JM, DeLong L, et al. Mutations in a signalling pathway. Nature. 2005 Aug 11;436(7052):792.
79. Alves-Guerra MC, Ronchini C, Capobianco AJ. Mastermind-like 1 is a specific coactivator of β-catenin transcription activation and is essential for colon carcinoma cell survival. Cancer Res. 2007 Sep 15;67(18):8690-8.
80. Meng RD, Shelton CC, Li YM, Qin LX, Notterman D, Paty PB, et al. γ-Secretase inhibitors abrogate oxaliplatin-induced activation of the Notch-1 signaling pathway in colon cancer cells resulting in enhanced chemosensitivity. Cancer Res. 2000 Jan 15;69(2):573-82.
81. Yao J, Duan L, Fan M, Yuan J, Wu X. Notch1 induces cell cycle arrest and apoptosis in human cervical cancer cells: involvement of nuclear factor kappa B inhibition. Int J Gynecol Cancer. 2007 Mar-Apr;17(2):502-10.

82. Qi R, An H, Yu Y, Zhang M, Liu S, Xu H, et al. Notch1 signaling inhibits growth of human hepatocellular carcinoma through induction of cell cycle arrest and apoptosis. Cancer Res. 2003 Dec 1;63(23):8323-9.
83. Varnat F, Duquet A, Malerba M, Zbinden M, Mas C, Gervaz P, et al. Human colon cancer epithelial cells harbour active HEDGEHOG-GLI signalling that is essential for tumour growth, recurrence, metastasis and stem cell survival and expansion. EMBO Mol Med. 2009 Sep;1(6-7):338-51.
84. Akiyoshi T, Nakamura M, Koga K, Nakashima H, Yao T, Tsuneyoshi M, et al. Gli1, downregulated in colorectal cancers, inhibits proliferation of colon cancer cells involving Wnt signalling activation. Gut. 2006 Jul;55(7):991-9.
85. Neiman P, Kimmel R, Icreverzi A, Elsaesser K, Bowers S, Burnside J, et al. Genomic instability during Myc- induced lymphomagenesis in the bursa of Fabricius. Oncogene. 2006 Oct 12;25(47):6325-35.
86. Prochownik EV. c-Myc: linking transformation and genomic instability. Curr Mol Med. 2008 Sep;8(6):446-58.
87. Kuzyk A, Mai S. c-MYC-induced genomic instability. Cold Spring Harb Perspect Med. 2014 Apr 1;4(4):a014373.
88. Felsher DW, Bishop JM. Transient excess of MYC activity can elicit genomic instability and tumorigenesis. Proc Natl Acad Sci USA.1999 Mar 30;96(7):3940-4.
89. Rockwood LD, Torrey TA, Kim JS, Coleman AE, Kovalchuk AL, Xiang S, et al. Genomic instability in mouse Burkitt lymphoma is dominated by illegitimate genetic recombinations, not point mutations. Oncogene. 2002 Oct 17; 21(47): 7235-40.
90. Silva AG, Graves HA, Guffei A, Ricca TI, Mortara RA, Jasiulionis MG, et al. Telomere-centromere-driven genomic instability contributes to karyotype evolution in a mouse model of melanoma. Neoplasia. 2010 Jan;12(1):11-9.
91. Aguilera A, Gómez-González B. Genome instability: a mechanistic view of its causes and consequences. Nat Rev Genet. 2008 Mar;9(3):204-17.
92. Rebucci M, Michiels C. Molecular aspects of cancer cell resistance to chemo-therapy. Biochem Pharmacol. 2013 May 1;85(9):1219-26.
93. Porta C, Paglino C, Mosca A. Targeting PI3K/ Akt/ mTOR signaling in cancer. Front Oncol. 2014 Apr 14;4:64.
94. Chang L, Graham P, Hao J, Ni J, Bucci J, Cozzi P, et al. Acquisition of epithelial-mesenchymal transition and cancer stem cell phenotypes is associated with activation of the PI3K/Akt/mTOR pathway in prostate cancer radioresistance. Cell Death Dis. 2013 Oct 24;4(10):e875.
95. Zhang X, Ai Z, Chen J, Yi J, Liu Z, Zhao H, et al. Glycometabolic adaptation mediates the insensitivity of drug- resistant K562/ ADM leukaemia cells to adriamycin via the AKT-mTOR/c-Myc signalling pathway. Mol Med Rep. 2017 Apr;15(4):1869-76.