IJBCM
International Journal of Basic and Clinical Medicine
Uluslararası Temel ve Klinik Tıp Dergisi

Review / Derleme

CANCER STEM CELLS : SECRET OF FAILURE

Kanser Kök Hücreleri: Başarısızlığın Sırrı

Selçuk Şeber[1] ,Tarkan Yetişyiğit[1]

[1] Namık Kemal University Faculty of Medicine, Department of Medical Oncology

Abstract

Although rapid responses or long disease free intervals can be retained in advanced stage cancer either by conventional treatment strategies or targeted therapies; cure is seldom if ever is obtained. Recent advances in tumor biology have succeeded in identifying a minor tumor cell subpopulation which have extraordinary capabilities for treatment resistance against various treatment strategies utilizing chemotherapeutic agents and radiotherapy. These cells carry stem cell like properties such as ability to initiate tumor by themselves, self renewal and ability to differentiate into heterogeneous cancer cell lines. In this review , survival and metastatic capabilities of cancer stem cells (CSCs), resistance mechanisms against chemotherapeutic agents are summarized . Novel therapeutic strategies developing against these resilient cell group such as agents specifically designed for targeting survival pathways and intracellular metabolism of CSCs are also discussed.

Key Words: Cancer stem cell (CSC); Drug Resistance; Therapy

Özet

İleri evre hastalıkta konvansiyonel tedavi rejimleri veya hedefe yönelik tedaviler ile yüksek yanıt oranları veya uzun süren hastalıksız sağkalım süreleri elde edilebilse bile şifa nadir elde edilen bir sonuçtur. Tümör biyolojisinde yakın zamanda ki gelişmeler sonucunda kemoterapi ve radyoterapiye dirençli az sayıda bulunan bir hücre alt grubunun varlığını ortaya çıkarmıştır. Bu hücre alt grubu tümör oluşturabilme, kendini yenileyebilme ve heterojen kanser hücre çeşitlerine başkalaşım gösterme kabiliyetleri gibi kök hücrelere benzer özellikler taşımaktadır.Bu derleme de kanser kök hücrelerinin metastaz yapabilme ve sağkalım özellikleri ile kemotöropatik ilaçlara direnç mekanizmaları değerlendirilmiştir..Bu hücre grubuna karşı geliştirilmekte olan kanser kök hücre hedefli yeni tedavi stratejileri de incelenmiştir.

Anahtar kelimeler: Kanser Kök Hücresi, İlaç Direnci,Tedavi

Introduction

Cancer is the result of genetic mutations resulting in activation or overexpression of proteins which are responsible for cell survival, and downregulation or loss of expression of other proteins which control cell cycle arrest and apoptosis[1]. Under normal conditions the genetic mutations favoring tumor formation are recognized and repaired by DNA control mechanisms. Cells who survive despite these genetic mutations are then recognized by the immune system and get destroyed by natural killer cells and other immune defense mechanism. However among the cancer cells those who succeed to evade innate immune response and also posses the ability to survive and proliferate and give rise to daughter cancer cells give rise to malignant tumors[2].

Cancer stem cell hypothesis

Recent advances in tumor biology have identified certain subpopulations in the tumor mass. Also called the hierarchical model, only a certain subpopulation of cells ; termed as cancer stem cells (CSCs); have the ability to initiate and sustain tumor growth .These subpopulation of cells also have heterogeneous differentiation and self renewal

Corresponding Author / Sorumlu Yazar:

Namık Kemal Üniversitesi Tıp Fakültesi Medikal Onkoloji BD
Değirmenaltı yerleşkesi, Tekirdağ
Email: esseber@nku.edu.tr

Article History / Makale Geçmişi:

Date Received / Geliş Tarihi: 07.09.2016
Date Accepted / Kabul Tarihi: 19.09.2016
Int J Basic Clin Med 2016; 4(2):101-8

capabilities. They are resistant to cytotoxic effects of chemotherapeutic agents and radiotherapy and can evade cytostatic intervention directed from targeted therapies. It is also hypothesized that they can evade detection from immune attacks directed against them since they share many of the stem cell markers that the normal adult stem cells carry on their surface[3].

CSC can originate from “recruited” adult stem cells already present in the normal tissue or it can originate from dedifferentiated cancer cells who by several genetic or epigenetic mutation gain stem like properties. Cancer cells generating from the same CSC can display different phenotypic and functional characteristics contributing to the heterogeneity of the tumor. This tumor model is termed as the hierarchical tumor model. In this model, cells forming the tumor mass are in a constant dynamic state where every cancer cell can transform into a CSC or vise versa depending on genetic and epigenetic changes occurring in response to environmental and intracellular changes[4].

Although CSC hypothesis continue to be debated in the oncology community, it answers some of the key aspects of treatment failure such as minimal residual disease, need for maintenance therapies, and occurrence of drug resistance in chemotherapy sensitive cancers. Current anticancer treatments can effectively kill differentiated cancer cells that form the bulk of the tumor load however they are far from eliminating the subpopulation of cancer initiating stem cells. Understanding how CSC survive and maintain their unlimited self renewal capabilities can lead the way to discover unique ways of targeting these cells

Definition and Characterization

CSCs were first reported in patients with acute myeloid leukemia, as CD34(+)/CD38(-) cells, which have the capacity to form leukemia in immunodeficient mice after xenotransplantation. Subsequently the presence of CSCs were reported from solid organ tumors[5]. In year 2006 American Association of Cancer Research, defined CSCs as a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that compose the tumor. These cells carry unlimited potential for proliferation and self renewal and differentiate into cancer cells which have a limited capacity for proliferation therefore they give rise to a heterogenic population of cancer cells forming a tumor mass[6]. CSCs also posses the ability to form functional blood vessels by transforming to endothelial progenitor cells thus can maintain the survivability of the tumor mass[7].

Chemotherapy and radiotherapy are treatment strategies which are designed to be effective in rapidly proliferating cells however CSCs during most of their lifetime remain in resting phase of the cell cycle which is termed as quiescence. This dormant stage of CSCs is a key feature for their resistance to chemotherapy[8]. In addition they express high levels of drug export systems which is another mechanism for resistance to conventional anticancer therapies[9].

The major obstacles that CSCs have to overcome during the metastatic process is avoiding immune attacks, surviving and replicating in different environments and avoiding programmed cell death upon cell detachment from extracellular matrix which is

termed as anoikis. Change in the type of integrins which are mediators between cell and extracellular matrix interaction are the key factor for escaping anoikis. In normal conditions integrins transduce signals from the extracellular matrix that regulate tissue homeostasis, survival and proliferation process.

However if anoikis is deregulated , cells detached from their environments gain ability to survive in suspension , free floating, conditions .CSCs by changing expression of their integrins can avoid initiation of cell death cascade[10].The most prominent change is downregulation of αvβ3 integrin expression which can be observed in CSCs across multiple types of cancer. In return another type of integrin, αvβ6 integrin , becomes overexpressed in CSCs. Integrin αvβ6 is usually expressed in organ developmental stages and cease to be expressed in normal adult cells. Integrin αvβ6 is strongly associated with activation of prosurvival pathways such as PI3K/AKT and PTEN that helps CSCs to avoid anoikis. Also switching to high expression αvβ6 integrin phenotype in CSCs promotes over secretion of metalloproteinase-3 (MMP-3) which directly increases the invasive properties of the cancer cell. which plays a key role in epithelial to mesenchymal transition[11].

Another feature of CSCs is overactivation of prosurvival pathways. PI3K/Akt is the main control pathway which many other accessory pathways are also regulated. Continuous activation of PI3K/Akt pathway is sustained by Ras mutations and functional loss of of the phosphatase and tensin homolog (PTEN) gene which is one of the most important tumor suppressor genes. Continuous Akt activation results in inhibition of apoptotic proteins such as procaspase -9, increases ATP binding cassette transporters, and results in overexpression of stem cell markers such as CD133[12]

Identification

Currently golden standard for identifying CSCs has not been established. One of the methods for identification of CSCs is detecting coexpression of various CSC markers in the membrane or cytoplasm of cancer cells. The markers specific for CSCs can vary according to the type or origin of the cancer cells. This makes it extremely difficult to determine universal stem cell markers .

The discovery of tumor initiating cells were first reported in acute myeloid leukemia. It was observed that cells expressing CD34+/CD38- adhesion marker phenotypes were able to initiate tumors in immunodeficient mice when transplanted. However cells lacking this phenotype were unable to initiate tumors when transplanted. Other studies also confirmed that only a subset of cancer cells with certain cell surface marker expressions had tumor initiating characteristics.

CSCs can be recognized by their expression of embryonic stem cell markers such as NANOG, SOX2, SALL4, and OCT4 . All of these markers are associated with pluripotency, evasion of apoptosis and invasion. Interestingly it has been hypothesized that there can be different subsets of CSCs inside the tumor mass which are coexpressing different sets of cell surface markers. CSCs express various CSC markers such as CD133, CD44, ESA, CXCR4, and nestin. Combinations of these and many other cell surface markers

are being elucidated to categorize CSCs in different solid organ tumors[13,14].

CD 133 a membrane protein commonly expressed by most of the CSCs. CD133 can be used for selecting purposes. The exact role of CD133 is not yet clearly identified. CD 133 expressing lung cancer cells are found to be the only tumorigenic subpopulation when transplanted to immunodeficient mice. CD133 overexpression subpopulation of cells also increased expression of genes associated with chemoresistance such as ABCG2 and CXCR4 after exposure to chemotherapeutics[15].

Nestin expression is commonly observed in progenitor cells during early nervous system development.. Nestin is responsible for formation of intermediate filaments in the cytoplasm and plays role in cytoplasmic reorganization taking place during mitosis. Its expression is increased in actively proliferating ductal structures and newly formed capillary endothelial cells. Increased nestin expression is observed in many different solid organ tumors and its presence is correlated with poor overall survival[16].

CSCs are identified by their increased efflux capacity of the Hoechst dye and these cells are also termed as side population (SP) cells. Along with cell surface markers and ability to form spheres in cell cultures , high drug efflux capacity is also used as a detection method for CSCs . High expression of proteins belonging to the ATP-binding (ABC) cassette transporter family such as P-glycoprotein is responsible for this feature[17]

Since cell surface marker phenotypes of all CSCc are yet to be defined other identification mechanisms are still under continuous evaluation. One very useful method is the sphere forming cultures. In vitro studies have shown that while ordinary cancer cells cannot proliferate in low attachment dishes, cells that carry stem cell properties can form spheres, floating colonies, in these environmental conditions. CSCs can proliferate without a need for cell attachment. In cell cultures, CSCs are selected by their ability to form floating spheres in low attachment mediums[18].

Resistance to therapy and Epithelial Mesenchymal Transition

One of the key features playing role in chemotherapy resistance in CSCs in epithelial mesenchymal transition (EMT) process. By EMT process, CSCs gain mesenchymal cell-like features. Also cells undergoing transition gain the ability to invade neighboring tissues and enter the circulation and travel to distant organs for formation of metastatic lesions. Interestingly once they reach the target organ CSCs can perform mesenchymal–epithelial transition (MET) which by the end of this process the cells gain epithelial functions[19].

Epithelial mesenchymal transition (EMT) is normally activated during embryogenesis and wound healing. By this transition cells can attach to neighboring environment and migrate. During EMT CSCs become resistant to anoikis and by interrelated processes also activate pro survival pathways such as PI3K/Akt and overexpress anti apoptotic genes such as Bcl-2. EMT is thought to occur by controlling gene expression which is named as alternative splicing. messenger RNA which will produce the specific protein can be changed by including or excluding exons from the same gene responsible for coding . Alternative splicing enables CSCs to produce

mesenchymal type proteins during EMT which give them features specific to mesenchymal cells[20]. One of the other mechanisms for drug resistance observed in these type of cells is the abundance of ABC transporters which are responsible for drug efflux process. Cells overexpressing stem cell markers are all expressors of ATPase transporters which quickly decrease the drug concentrations inside the CSCs[21].

Another key event in EMT process is activated Src activity commonly observed in CSCs. This activation results in endocytosis of E-cadherin and overexpression of N-cadherin. Switch to N cadherin expression results in signals which activates PI3K pathway and resistance to programmed cell death [22]. Other important regulators which take role in EMT process are transcription factors Snail, NF-kB and HIF 1/2. All of these transcription factors work in an orchestrated manner to increase the CSCs ability for migration, invasion, and resistance to apoptosis and anoikis, repression of E cadherin expression, downregulation of PTEN activation and sustained activation of PI3K/Akt pathway.

Quick proliferation and mass formation characteristics of tumor cells create hypoxic regions within the tumor environment. Hypoxia inducible factors (HIF) is a transcription factor which becomes upregulated in hypoxic conditions of tumor microenvironment . Increased HIF1 alpha is responsible for the accentuation of the EMT process and overactivation of prosurvival pathways such as Notch 1 signaling which enables CSCs to resist apoptosis and enable them to sustain proliferation and invasion capabilities even in this unfavourable environment[23].

Like many other pathways which play important roles in tumorigenesis and impairment of organ injury , hedgehog pathway (Hh) activation holds a critical role in stemness of cancer cells because it controls expression of GLI proteins.GLI proteins are transcription factors which their activation lead to production of Hh target proteins. These targets can be listed as but not limited to Notch signaling pathways, BCL-2 and the ATP-binding cassette transporter family members. These pathways are necessary for stem cell viability and capability for drug resistance[24].

Future Treatment Strategies

So far agents targeting CSCs has not gained wide clinical access and developing treatments are largely confined to animal models and preclinical studies.

Targeting proteins which are the products of alternative splicing in EMT can be an effective treatment. Inhibition of EMT process will disrupt formation of metastasis and also hinder chemotherapy resistance. One such factor is fibroblast growth factor receptor (FGFR)-2 . (FGFR)-2 IIIc is a mesenchymal variant of FGFR-2 and it is produced during the EMT process . It has been shown in several preclinical studies that inhibition of mesenchymal variant of FGFR-2 inhibits metastasis formation and slows proliferation rate of cancer cells. It is also possible to intervene with the alternative splicing and promote epithelial variants of the proteins instead of the mesenchymal ones by RNA transfection methods [25].There are also several FGFR inhibitors being developed for the clinical setting which have shown effectiveness in preclinical models [26].

Another method in fight against CSCs is systemic administration of small interfering RNA (si-RNA) with the aim of selectively silencing chosen gene expressions. This revolutionary treatment enables targeting genetic machinery that is necessary for the production of specific proteins which play a key role in stemness of the cancer cells. One of these target proteins is nestin. In vitro studies have shown that silencing nesting expression by siRNA can decrease the proliferation rate of cancer cells and in addition increase E-cadherin production by the cells. This indicates nestin targeting can reverse the EMT process and interfere with the occurrence of stem like features [27] .

Overexpression of Aldehyde Dehydrogenase (ALDH) is another feature of CSCs. Lunasin, a soy product derivative has been reported to have significant inhibitory effect for ALDH both in vivo and in vitro. Lunasin has been shown to induce CSCs to a more differentiated phenotype with less self renewal capabilities[28].

Another agent that is evaluated for targeting ALDH is disulfiram. Disulfiram is an aldehyde dehydrogenase inhibitor which is already used as an alcohol aversion therapy in the clinic. Disulfiram/copper complex has been shown to inhibit the tumor initiating and proliferative capacity of CSCs of non small cell lung cancer origin both in vivo and in mice xenograft models. by inhibiting expression of stem cell transcription factors Nanog and Oct-4[29].

Targeting the Hedgehog pathway is another target for deactivation of CSCs. Hedgehog pathway which is responsible for sustained stemness during embryonic developmental stages becomes overactivated by genetic mutations in CSCs. New generation hedgehog pathway inhibitors such as vismodegib have found their way to phase I and II clinical studies and so far have shown promising results[30].

Targeting the functions of ABC transporter proteins is a future aim for anti CSC treatments Third generation anti ABC transporter proteins such as zosuquidar (LY335979) and tariquidar (XR9576) have less side effects and increased affectivity compared to previous generation inhibitors and are being included in the clinical trials. Of interest a plant derivative, deoxyschizandrin has been shown to inhibit P glycoprotein and reverse resistance to chemotherapeutic drugs such as adriamycin and vincristine[31].

Multitargeting of growth pathways that CSCs are dependent for survival is a feasible approach. In a study by Yoon et. al. concurrent use of HIF 1 alpha inhibitor (doxorubicin) with anti vascular endothelial growth factor (VEGF) inhibitor therapy(pazopanib) plus hypoxia activated chemotherapy (evofosfamide) in a murine model have demonstrated selective killing of CSCs in vivo. When angiogenesis is inhibited by VEGF inhibitors HIF 1 alpha pathway is utilized as a backup pathway for survival in tumor initiating cells. CSCs are also believed to favor hypoxic environments for growth. Therefore simultaneous blockage of VEGF and HIF pathways in addition to using drugs more active in hypoxic conditions was evaluated. This multimodal therapy have resulted in depletion of CD133 cells and reduced tumor burden in xenografted immunodeficient mice[32].

Conclusion

The CSC hypothesis helps to our understanding of tumor biology and anti cancer treatment. These cells carry several characteristics that make cancer a difficult disease to treat such as tumor initiation, ability to metastasize, multipotency and treatment resistance. Treatments aimed at targeting CSCs directly or inhibition of normal cancer cells to gain stemness carry great potential to cure this deadly disease. However CSCs are a heterogeneous population and they are in a dynamic state of transforming in and out of epithelial or mesenchymal states Therefore combination therapies targeting several survival pathways concurrently and have ability to be toxic for cells with proliferating and quiescent stages carry a greater potential for effective control of the disease.

References

1. Bertram JS. The molecular biology of cancer. Mol Aspects Med. 2000;21(6):167-223. http://www.ncbi.nlm.nih.gov/pubmed/11173079. Accessed August 31, 2016.
2. Bruttel VS, Wischhusen J. Cancer stem cell immunology: key to understanding tumorigenesis and tumor immune escape? Front Immunol. 2014;5:360. doi:10.3389/fimmu.2014.00360.
3. Joshi P, Kooshki M, Aldrich W, et al. Expression of natural killer cell regulatory microRNA by uveal melanoma cancer stem cells. Clin Exp Metastasis. August 2016. doi:10.1007/s10585-016-9815-9.
4. Nagai Y, Kawahara M, Hishizawa M, et al. T memory stem cells are the hierarchical apex of adult T-cell leukemia. Blood. 2015;125(23):3527-3535. doi:10.1182/blood-2014-10-607465.
5. Yamazaki J, Mizukami T, Takizawa K, et al. Identification of cancer stem cells in a Tax-transgenic (Tax-Tg) mouse model of adult T-cell leukemia/lymphoma. Blood. 2009;114(13):2709-2720. doi:10.1182/blood-2008-08-174425.
6. Song Y, Sun B, Hao L, et al. Elevated eukaryotic elongation factor 2 expression is involved in proliferation and invasion of lung squamous cell carcinoma. Oncotarget. August 2016. doi:10.18632/oncotarget.11298.
7. Yao L, Zhang D, Zhao X, et al. Dickkopf-1-promoted vasculogenic mimicry in non-small cell lung cancer is associated with EMT and development of a cancer stem-like cell phenotype. J Cell Mol Med. 2016;20(9):1673-1685. doi:10.1111/jcmm.12862.
8. Li L, Bhatia R. Stem cell quiescence. Clin Cancer Res. 2011;17(15):4936-4941. doi:10.1158/1078-0432.CCR-10-1499.
9. Guan G-F, Zhang D-J, Zheng Y, et al. Significance of ATP-binding cassette transporter proteins in multidrug resistance of head and neck squamous cell carcinoma. Oncol Lett. 2015;10(2):631-636. doi:10.3892/ol.2015.3359.
10. Yang M-H, Imrali A, Heeschen C. Circulating cancer stem cells: the importance to select. Chin J Cancer Res. 2015;27(5):437-449. doi:10.3978/j.issn.1000-9604.2015.04.08.
11. Seguin L, Desgrosellier JS, Weis SM, Cheresh DA. Integrins and cancer: regulators of cancer stemness, metastasis, and drug resistance. Trends Cell Biol. 2015;25(4):234-240. doi:10.1016/j.tcb.2014.12.006.
12. Xia P, Xu X-Y. PI3K/Akt/mTOR signaling pathway in cancer stem cells: from basic research to clinical application. Am J Cancer Res. 2015;5(5):1602-1609. http://www.ncbi.nlm.nih.gov/pubmed/26175931. Accessed September 1, 2016.
13. Schulenburg A, Blatt K, Cerny-Reiterer S, et al. Cancer stem cells in basic science and in translational oncology: can we translate into clinical application? J Hematol Oncol. 2015;8:16. doi:10.1186/s13045-015-0113-9.
14. Lathia JD, Venere M, Rao MS, Rich JN. Seeing is believing: are cancer stem cells the Loch Ness monster of tumor biology? Stem Cell Rev. 2011;7(2):227-237. doi:10.1007/s12015-010-9194-6.
15. Bertolini G, Roz L, Perego P, et al. Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. Proc Natl Acad Sci U S A. 2009;106(38):16281-16286. doi:10.1073/pnas.0905653106.
16. Neradil J, Veselska R. Nestin as a marker of cancer stem cells. Cancer Sci. 2015;106(7):803-811. doi:10.1111/cas.12691.
17. Britton KM, Kirby JA, Lennard TWJ, Meeson AP. Cancer stem cells and side population cells in breast cancer and metastasis. Cancers (Basel). 2011;3(2):2106-2130. doi:10.3390/cancers3022106.
18. Pastrana E, Silva-Vargas V, Doetsch F. Eyes wide open: a critical review of sphere-formation as an assay

for stem cells. Cell Stem Cell. 2011;8(5):486-498. doi:10.1016/j.stem.2011.04.007.

19. Du B, Shim JS. Targeting Epithelial-Mesenchymal Transition (EMT) to Overcome Drug Resistance in Cancer. Molecules. 2016;21(7). doi:10.3390/molecules21070965.
20. Yang Z, Xie H, He D, Li L. Infiltrating macrophages increase RCC epithelial mesenchymal transition (EMT) and stem cell-like populations via AKT and mTOR signaling. Oncotarget. June 2016. doi:10.18632/oncotarget.9873.
21. Saxena M, Stephens MA, Pathak H, Rangarajan A. Transcription factors that mediate epithelial-mesenchymal transition lead to multidrug resistance by upregulating ABC transporters. Cell Death Dis. 2011;2:e179. doi:10.1038/cddis.2011.61.
22. Lotz-Jenne C, Lüthi U, Ackerknecht S, et al. A high-content EMT screen identifies multiple receptor tyrosine kinase inhibitors with activity on TGFβ receptor. Oncotarget. 2016;7(18):25983-26002. doi:10.18632/oncotarget.8418.
23. Joseph J V, Conroy S, Pavlov K, et al. Hypoxia enhances migration and invasion in glioblastoma by promoting a mesenchymal shift mediated by the HIF1α-ZEB1 axis. Cancer Lett. 2015;359(1):107-116. doi:10.1016/j.canlet.2015.01.010.
24. Fukushima N, Minami Y, Kakiuchi S, et al. Small-molecule Hedgehog inhibitor attenuates the leukemia-initiation potential of acute myeloid leukemia cells. Cancer Sci. July 2016. doi:10.1111/cas.13019.
25. Matsuda Y, Yoshimura H, Suzuki T, Uchida E, Naito Z, Ishiwata T. Inhibition of fibroblast growth factor receptor 2 attenuates proliferation and invasion of pancreatic cancer. Cancer Sci. 2014;105(9):1212-1219. doi:10.1111/cas.12470.
26. Yan W, Wang X, Dai Y, et al. Discovery of 3-(5'-Substituted)-Benzimidazole-5-(1-(3,5-dichloropyridin-4-yl)ethoxy)-1H-indazoles as Potent Fibroblast Growth Factor Receptor Inhibitors: Design, Synthesis, and Biological Evaluation. J Med Chem. 2016;59(14):6690-6708. doi:10.1021/acs.jmedchem.6b00056.
27. Matsuda Y, Ishiwata T, Yoshimura H, Hagio M, Arai T. Inhibition of nestin suppresses stem cell phenotype of glioblastomas through the alteration of post-translational modification of heat shock protein HSPA8/HSC71. Cancer Lett. 2015;357(2):602-611. doi:10.1016/j.canlet.2014.12.030.
28. Shidal C, Al-Rayyan N, Yaddanapudi K, Davis KR. Lunasin is a novel therapeutic agent for targeting melanoma cancer stem cells. Oncotarget. August 2016. doi:10.18632/oncotarget.11554.
29. Liu X, Wang L, Cui W, et al. Targeting ALDH1A1 by disulfiram/copper complex inhibits non-small cell lung cancer recurrence driven by ALDH-positive cancer stem cells. Oncotarget. August 2016. doi:10.18632/oncotarget.11305.
30. Graham RA, Lum BL, Cheeti S, et al. Pharmacokinetics of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with locally advanced or metastatic solid tumors: the role of alpha-1-acid glycoprotein binding. Clin Cancer Res. 2011;17(8):2512-2520. doi:10.1158/1078-0432.CCR-10-2736.
31. Gupta PB, Onder TT, Jiang G, et al. Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell. 2009;138(4):645-659. doi:10.1016/j.cell.2009.06.034.
32. Yoon C, Chang KK, Lee JH, et al. Multimodal targeting of tumor vasculature and cancer stem-like cells in sarcomas with VEGF-A inhibition, HIF-1α inhibition, and hypoxia-activated chemotherapy. Oncotarget. June 2016. doi:10.18632/oncotarget.10212.