International Journal of Research in Pharmacology & Pharmacotherapeutics

ISSN Print: 2278 – 2648 ***IJRPP |Vol.3 | Issue 4 | Oct-Dec-2014***
ISSN Online: 2278-2656 ***Journal Home page: www.ijrpp.com***

Review article ***Open Access***

A Review on development of anti cancer drugs targeting cancer stem like cells

***V Hrishi, V Sekar, R Sambathkumar**

J.K.K.Nattraja College of Pharmacy Kumarapalayam, Tamil Nadu- 638183

***Corresponding author: V Hrishi.**

E-mail id: hrishivarayathu@gmail.com

ABSTRACT

The existence of subpopulations of cells with self-renewing capacity that are responsible for tumour maintenance, chemo resistance and metastasis, called cancer stem-like cells (CSLC) has been demonstrated in a wide variety of solid and hematopoietic malignancies and has attracted a great attention as novel cellular targets for anticancer therapy[1]. Now cancer stem cells are considered as the hallmarks of the cancer. Various pharmaceuticals have started to identify several cancer stem cells and drugs which are effective to hit it. But screens for agents that specifically kill epithelial cancer stem cells (CSCs) have not been possible due to the rarity of these cells within tumour cell populations and their relative instability in culture[3,4]. Even though certain compounds have been discovered as a targeted therapy such as salinomycin. From various researches it has been found out that salinomycin treatment results in the loss of expression of breast CSC genes[11,12]. Since conventional therapies have been designed to target proliferating cells, slow growing CSCs may be relatively more resistant than faster growing tumour bulk. Cancer stem cells resistance to standard chemotherapeutics has been reported in human leukemia, malignant melanoma, brain, breast, pancreatic and colorectal cancers. Cancer stem cells radio resistance has been reported in brain and breast cancers[15, 26]. Thus, if CSCs are selectively targeted by new stem line therapeutics, tumour size should be greatly reduced. This study is a comprehensive review of the various approaches to target the cancer stem cells and their effectiveness.

Keywords: Cancer stem cell, Signal pathways, Drug resistance.

INTRODUCTION

From various researches they have been found out that the existence of subpopulations of cells with self-renewing capacity that are responsible for tumour maintenance, chemo resistance and metastasis, called cancer stem-like cells[1-9]. A number of them are over expressed in various cancer types and play a role in the regulation of various cancer-promoting processes (proliferation, apoptosis, migration, epithelial-mesenchymal transition, hypoxia and DNA damage response), and may serve as anticancer drug targets. However, so far their role in CSLC biology has not been systematically studied. CSCs are resistant to many current cancer treatments, including chemo-

and radiation therapy. This suggests that many cancer therapies, while killing the bulk of tumour cells, may ultimately fail because they do not eliminate CSCs, which survive to regenerate new tumour. Thus, a CSC-targeted drug delivery strategy to eliminate CSCs is a desirable approach for developing a more effective anticancer therapy. Certain pharmaceutical companies such as Oncomed and Verastem are developing drug therapies that target cancer stem cells[15-19]. They have found out certain candidates and they are in clinical development now. Thus it is very clear that this will be the future therapy for cancer.

Cancer stem cells and their distribution

Cancer stem cells have been identified in a growing number of hematopoietic cancer and solid tumours and are typically recognized by virtue of the expression of cell surface markers. These cells have been isolated from the bulk tumour population by the expression pattern of cell surface proteins (e.g., CD24, CD44, and CD133) and cellular activities, such as the efflux of Hoechst dye or aldehyde dehydrogenase activity by flow cytometry and/or fluorescence activated cell sorting (FACS).

The identification of markers that allow the prospective isolation of CSCs from whole tumour tissues will lead to the understanding of important biological properties of CSCs and provide the possibility to target them. Besides the CD133, CD44, ALDH, and ABCG2, there have been many other cell surface proteins been identified as the marker of many tumours in the recent years (Table 1). Although CD133, CD44, ALDH, ABCG2 and other proteins have been used to identify cancer stem cells, it is still important to clarify cellular and signalling functions of markers themselves.

Biomarkers of the cancer stem cells not only the tools to which were used to identify the cancer stem cells, but also might become the target of many drugs which were developed to cure cancer. In the series research of those proteins, they are proved to be associated with tumour progression, maintenance and metastasis. At present, CSC markers must be clearly defined for each tissue, and investigation is needed to determine whether variations between tumours can be used as indicators of sensitivity to therapy[13-28]. However, no specified drugs have been developed to target those cell markers, monoclonal antibodies and siRNA and /or shRNA could sensitize cells to traditional therapy or even cure cancer. Finally, clarifying cellular and signalling functions of markers themselves will lead to more therapeutic options to destroy tumour cells that have the ability to repopulate.

Table: 1

Marker	Tumour type	Marker	Tumour type
	Brain		Breast
	Prostate		Lung
	Pancreas		Head and Neck
CD133	Melanoma	**ALDH**	Colon
	Colon		Liver
	Liver		Pancreas
	Lung		Prostate
	Ovary		Gastric
	Colon		
	Breast		
CD44	Prostate	**ABCB5**	Melanoma
	Pancreatic		
	Pancreas		
	Lung		
	Brain		
	Prostate		T acute lymphoblastic
ABCG2	Liver	**CD90**	gliomas
	Ovarian		Liver
	Retinoblastoma		
	Limbal epithelium		

Targeting signal pathways[1-16]

Tumour formation and progression occur through a range of defects that develop both within and outside the cancer cell. Defects in cell-signaling pathways allow cancer cells to alter their normal programmes of proliferation, transcription, growth, migration, differentiation and death. Some researchers have suggested that signal path ways' disorder or excessive activation may lead to the tumour genicity. Understanding the mechanisms that underlie the self-renewal behaviour of CSCs is of greatest importance for discovery and development of anticancer drugs targeting CSCs. During those pathways, Wnt, Notch and Hedgehog signalling pathways may play an important role in the recurrence and maintenance of cancer stem cell.

CSCs are characterized by tumourigenic properties and the ability to self-renew, form differentiated progeny, and develop resistance to therapy. CSCs use many of the same signaling pathways that are found in normal stem cells, such as Wnt, Notch, and Hedgehog (Hh). The origin of CSCs is not fully understood, but data suggest that they originate from normal stem or progenitor cells, or possibly other cancer cells. Therapeutic targeting of both CSCs and bulk tumour populations may provide a strategy to suppress tumour re growth. Development of agents that target critical steps in the Wnt, Notch, and Hh pathways will be complicated.

Wnt is a group of secreted signalling proteins that bind receptor molecules on the surface of target cells. The strongest evidence of the importance of the Wnt pathway to CSC biology has been reported in myeloid leukemias and it is also been reported to be implicated in the maintenance of CSCs of melanoma, breast, colon, liver and lung cancers. β- Catenin the essential mediator of Wnt signaling is involved in two distinct functions in the cell, depending on its cellular localization. The membrane-localized β-Catenin is sequestered by the epithelial cell– cell adhesion protein E-cadherin to maintain cell–cell adhesion and the cytoplasmic accumulation of β-Catenin and its subsequent nuclear translocation eventually leads to activation of Wnt target genes such as c-Jun, c-Myc, fibronectin and cyclin D1. Activated Wnt/β- Catenin signaling is a key feature of epithelial cancers and is perceived as critical for metastasis and epithelial-mesenchymal transitions (EMT).

A broad spectrum of compounds seems useful to specifically modulate Wnt/β-Catenin signals. Those drugs may also help to eliminate drugs-resistant CSC, which is thought to be responsible for tumour relapse and metastasis. Furthermore, newly created inhibitors of Wnt/β-Catenin signaling have just entered preclinical trials, such as monoclonal antibodies and small interfering RNAs against Wnt1/2, WIF1 and SFRPs, PRI-724 and CWP232291. The simultaneous discovery of Tankyrase (Tnks) enzymes as critical regulators of Axin and β-Catenin protein levels can also be useful for identifying newer drugs. The compound XAV939 antagonizes Wnt signalling via stimulation of β catenin degradation and stabilization of axin.

Notch signalling pathway is a highly conserved developmental pathway, which plays a critical role in cell-fate decision, tissue patterning and morphogenesis. There are four human Notch receptors that consist of an extracellular peptide containing epidermal growth factor receptor like repeats and a trans membrane peptide. Notch 1 and Notch 2 are the most ubiquitously distributed whereas Noth 2 and Notch 4 are more specifically expressed in vascular smooth muscle and endothelial cells.

The Notch pathway plays an important role in maintenance of the stem cell in glioblastoma, breast cancer stem cells and some other tumour stem cells. Since the activation of Notch signaling can up regulate several factors that in turn transmit bidirectional signals among cancer cells expressing both ligands and receptors and it can also transmit signals among cancer, stromal and endothelium cells. In addition to this researches found out that it is the major pathway for the production of CSCs in ovarian cancer. In contrast, γ-secretase inhibitor(GSI), a Notch pathway inhibitor, depletes CSCs and increases tumour sensitivity to platinum. Similarly, a Notch3 siRNA knockdown increases the response to platinum therapy, further demonstrating that modulation of tumour chemo sensitivity by GSI is Notch specific. Most importantly, the Cisplatin/GSI combination is the only treatment that effectively

eliminates both CSCs and the bulk of tumour cells, indicating that a dual combination targeting both populations is needed for tumour eradication.

Standard chemotherapy in ovarian cancer results in tumour cytoreduction but infrequently results in a cure. It is well established that CSCs have key characteristics allowing them to survive standard cancer chemotherapy and radiation therapy. Recent studies indicate that CSCs are a major source of tumour development and resistance to chemotherapy.

Notch is a conserved pathway that has been implicated in the maintenance of tissue homeostasis by regulation of self-renewal and cell-fate determination in normal stem cells and early progenitors. Recent studies, including TCGA data, have found that the Notch pathway is misregulated in ovarian cancer. In addition, Notch3 gene copy number is increased and correlates with aggressive tumour behaviour and poor prognosis in ovarian serous adenocarcinomas.

The Notch signaling pathway is required in both non-neoplastic neural stem cells and embryonal brain tumours, such as medulloblastoma, which are derived from such cells. Researchers investigated the effects of Notch pathway inhibition on medulloblastoma growth using pharmacologic inhibitors of alpha secretase. Notch blockade suppressed expression of the pathway target Hes1and caused cell cycle exit, apoptosis, and differentiation in medulloblastoma cell lines. They found that Notch blockade reduced the CD133-positive cell fraction almost 5-fold and totally abolished the side population, suggesting that the loss Of tumour forming capacity could be due to the depletion of stem-like cells. Notch signaling levels were higher in the stem-like cell fraction, providing a potential mechanism for their increased sensitivity to inhibition of this pathway. This study observed that apoptotic rates following Notch blockade were almost 10-fold higher in primitive nestin-positive cells as compared with nestin-negative ones. Stem-like cells in brain tumours thus seem to be selectively vulnerable to agents inhibiting the Notch pathway.

The Hedgehog (Hh) signaling pathway was initially identified in Drosophila as a critical mediator of segmental patterning during embryonic development, and it regulates the proliferation, migration, and differentiation of target cells in a spatial, temporal, and concentration dependent manner. Emerging data from many tumours including glioblastoma, pancreatic adenocarcinoma, breast cancer, multiple myeloma, and chronic myeloid leukemia (CML) have suggested that Hh signaling regulates cancer stem cells. The mechanism of Hh signalling pathway is it comprises three main components the Hh ligand; a trans membrane receptor circuit composed of the negative regulator Ptc, an activator Smo, and finally a cytoplasmic complex that regulates the Ci or Gli family of transcriptional effectors. Ptc, a twelve-pass membrane protein binds Hh ligand, and in the absence of ligand, Ptc interacts with and inhibits Smo, a seven pass membrane protein. When Hh binds Ptc, its interactions with Smo are altered, leads to Ci/Gli protein entering the nucleus and acting as a transcriptional activator for the same genes.

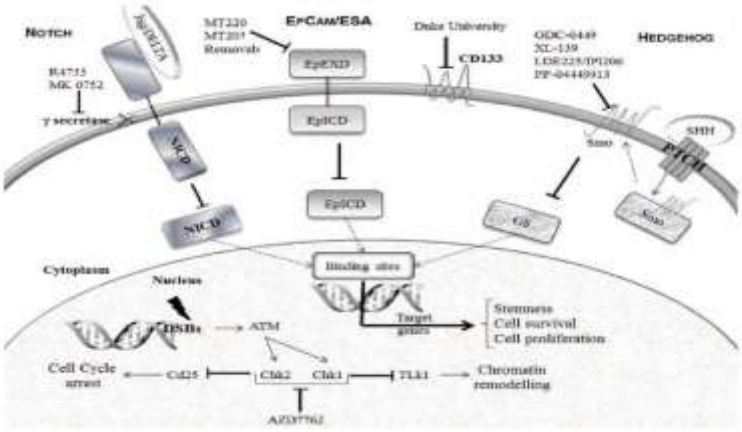


Figure 1: Potential drugs in clinical trials and cellular pathways targeted.

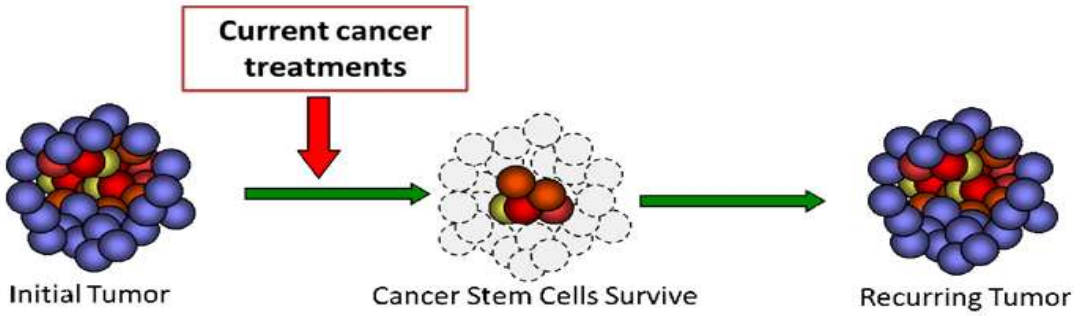
On-going cancer stem cells approaches

Several pharmaceutical companies and researchers are now carrying researches on identifying new and effective inhibitor of cancer stem cells. Verastem and Oncomed are recently published details on their candidates of CSCs inhibitors[5].

Vantictumab is a candidate of Oncomed and they passed the phase II clinical trials. Verastem also carrying on researches on so many CSC inhibitors.

Standard Approach

The CSCs within tumour have the capacity to regenerate the primary tumour. They also acquire characteristics that increase their invasive potential. The ability of CSCs to move to other sites in the body, combined with the capability to initiate a new tumour mass, may implicate CSCs as an important factor in the formation of metastases. Metastatic tumour sites are the cause of death in more than 90% of human cancer patients. Cancer stems cells have been found in many types of tumours, including leukemia, myeloma, breast, prostate, colon, brain, lung and other cancers.

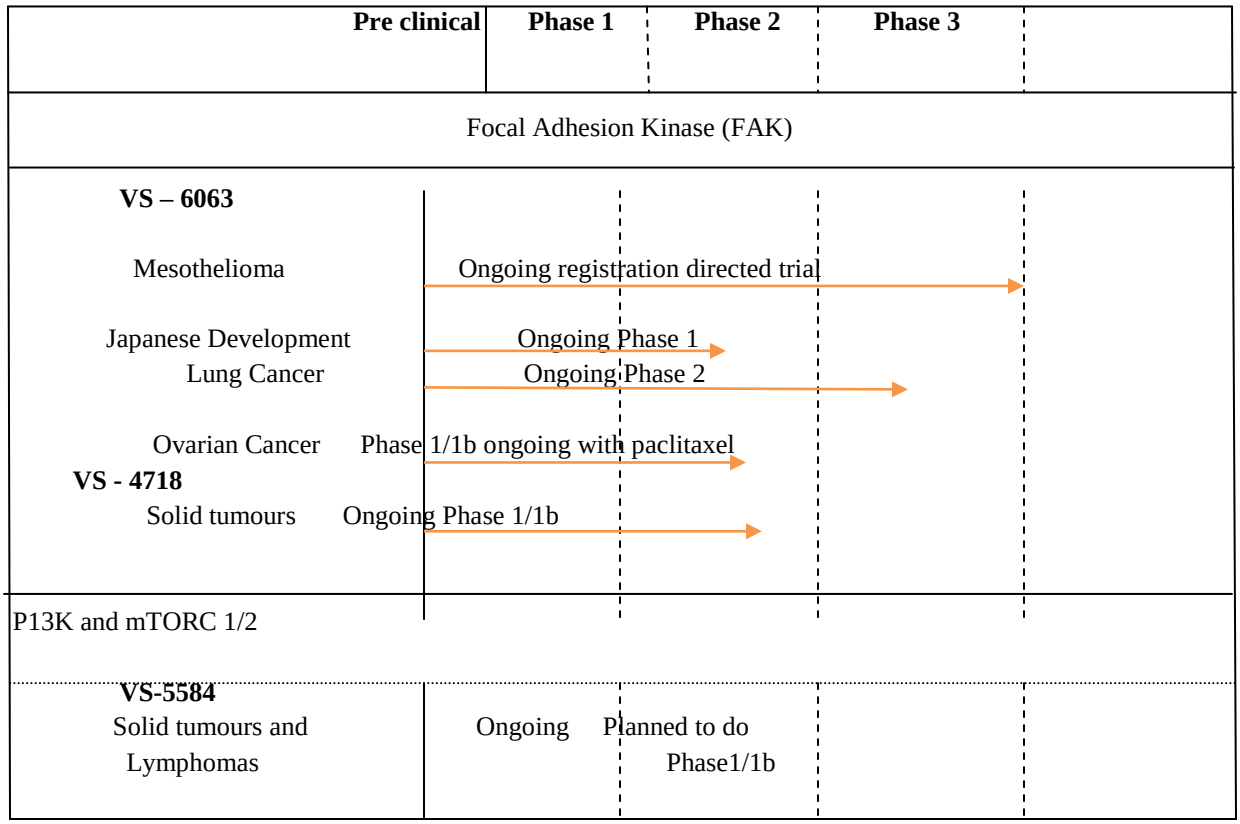


Current Approaches

Verastems describes that they are developed a system for the discovery of a new generation of cancer drugs that can selectively target CSCs and provide a strategy for treatment of cancer patients. This has been accomplished by developing novel methods to create large numbers of cells that stably reside within the cancer stem-cell state. By engineering tumour cells to stably transition to CSCs, Verastem has established high-throughput drug discovery systems that make it possible to identify and develop drugs specifically targeting CSCs[4-10].

Verastem is developing small molecule inhibitors of signaling pathways that are critical to cancer stem cell survival and proliferation: **FAK, PI3K/mTOR and Wnt (Fig 2).**

Figure 2. Verastems approaches



FAK INHIBITION PROGRAM[5-21]

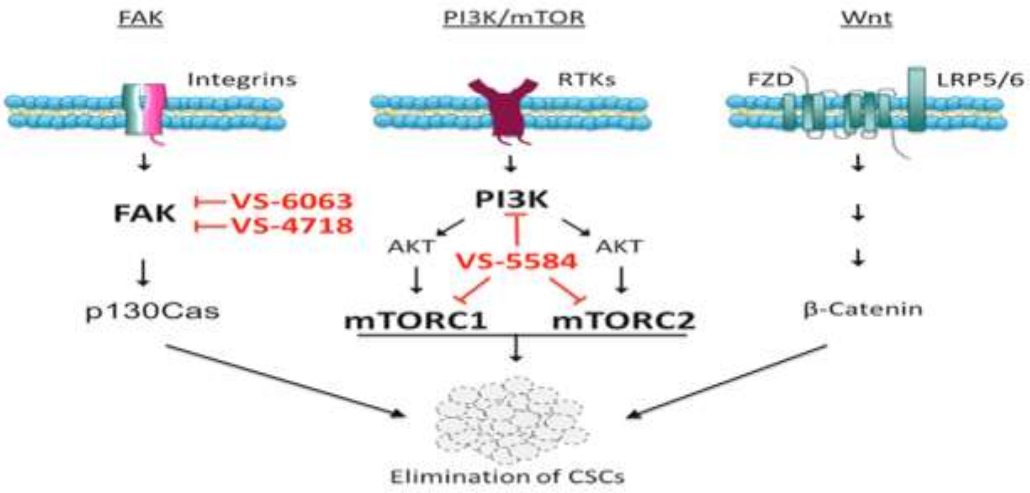
FAK expression is greater in many tumour types as compared to normal tissue, particularly in cancers that have high invasive and metastatic capability. FAK activity appears to be critical in disease progression and establishment of new tumours – hallmarks of cancer stem cells. VS- 6063 has a good safety profile and demonstrated initial signs of activity in a 46 patient, Phase 1 study, in advanced solid tumours.

Mesothelioma is a malignant tumour that most commonly occurs in the protective lining (the pleura) surrounding the lungs. Asbestos fibers are the cause of most cases (85%) of mesothelioma. Approximately 13,000 cases of the aggressive disease are diagnosed worldwide each year. Verastem is currently conducting the registration-directed COMMAND study of VS-6063 for patients with malignant pleural mesothelioma.

Ovarian cancer rapidly develops resistance to chemotherapy and there is no standard of care once resistance to platinum-based chemotherapy develops although treatment with the chemotherapeutic paclitaxel is often used. There are approximately 225,000 cases of ovarian cancer diagnosed each year worldwide. Initial signs of activity of VS-6063 were observed in Phase 1 testing. The use of VS-6063 in combination with paclitaxel paves the way for testing in additional solid tumours where treatment of paclitaxel is prescribed (e.g. breast and lung).

PI3K/MTOR inhibition program[6-11]

The PI3K/mTOR signaling pathway is a key regulator in cancer progression and the survival of cancer stem cells. Researchers identified the dual inhibition of both PI3K and mTORC1 and mTORC2 that can preferentially kill cancer stem cells. VS-5584 is a potent and selective small molecule inhibitor of PI3K and mTORC1 and mTORC2.



Vantictumab

This is an oncomed candidate in targeting cancer stem cells. Vantictumab is a monoclonal antibody that blocks canonical WNT/β-catenin signaling through binding of five Fzd receptors (1, 2, 5, 7, 8) at a conserved epitope within the extracellular domain. Mouse xenograft studies using minimally-passaged, patient-derived xenografts show that vantictumab impedes tumour growth, promotes differentiation and reduces CSCs in multiple tumour types and synergises with many chemotherapeutic agents. In nonclinical models, vantictumab modulates gene expression in tumour cells associated with stem cell and differentiation pathways and down-regulates Wnt pathway genes in the tumour and stroma. As such, vantictumab is a novel anti-cancer agent that inhibits tumour growth through direct actions on tumour cells, including CSCs, and effects on the stroma.

Wnt pathway genes (e.g., AXIN2), and stem cell and differentiation genes (e.g., BMP8B, GFI1) were found to be regulated in hair follicles by vantictumab treatment. In tumours, vantictumab inhibited Wnt target (e.g., AXIN2, TCF4), stem cell and EMT genes (HOXA2, ZEB2) and increased the expression of differentiation genes, including MUC4, MUC5B, and MUC2.

Mechanism of resistance[19-26]

Cancer stem cell is resistant to chemo- and radiation therapy, often lead to the failure of conventional therapy and result relapse. Frequent cancer recurrence may be due to the preferential killing of differentiated cells while leaving CSCs behind. A better understanding of the mechanisms that underlying CSCs resistance to treatment is necessary and may provide a more effective therapy to overcome the resistance. One potential modulator of CSC resistance to DNA targeting agents is the family of checkpoint kinases 1/2 (Chk1/2 kinases) and these kinases have higher basal and inducible activities in CSCs than in nonstem cells. An experiment showing that pancreatic CSCs could survive and expand after serial exposures to gemcitabine, this chemo resistance was overcome by the use of CD44 or ABC transporter inhibitors. There are some additional strategies to overcome therapeutic resistance during cancer treatment.

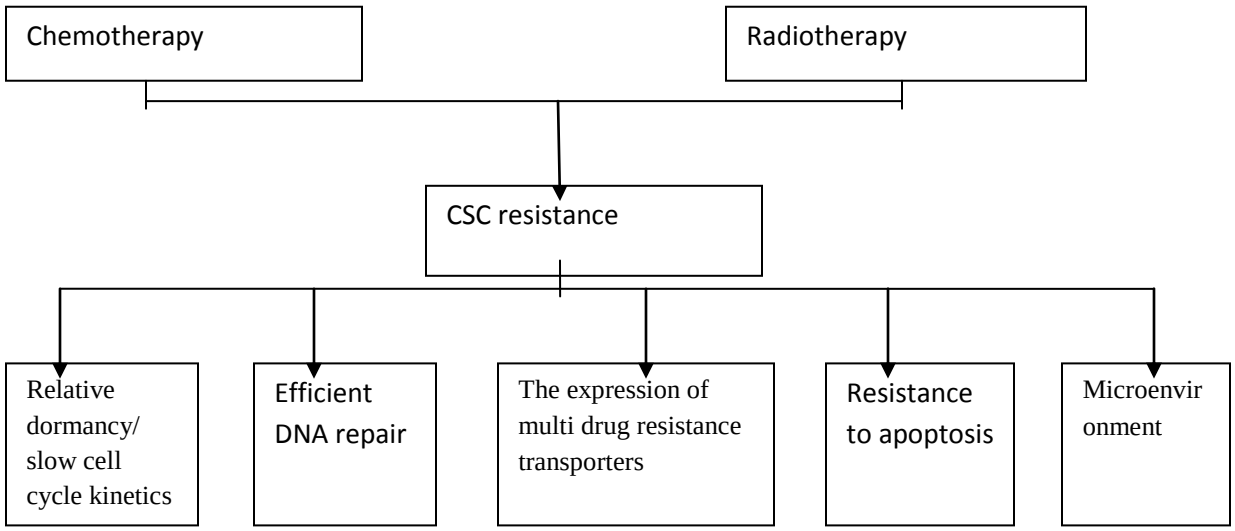


Fig: 3 - Mechanism leading to cancer stem cell resistance to chemo and radiation therapy

Even though there are some additional strategies to overcome therapeutic resistance during cancer treatment[1-4].

1. Concurrent therapy: It is now well established that combination therapy helps to prevent the development of cancer resistance, but for a select group of cancer types where a single pharmaceutically correctable mutation exists.
2. Surgical Resection following induction: theoretically, CSC-specific induction chemotherapies should offer an immediate reduction in CSC metastatic potential and reduce any haematogenous and lymphatic CSC micro metastases that would otherwise diminish the efficacy of surgical resection.

REFERENCES

[1] Yapeng Hu, Liwu Fu. Targeting cancer stem cells: a new therapy to cure cancer patients. Am J Cancer Res 2012;2(3):340-356.
[2] Gupta PB, Onder TT, et al. Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell. 2009 Aug 21; 138(4):645-59.
[3] Douglas H, Robert A. Hallmark of cancer: The next generation. Cell. Volume 144, Issue 5, 646-674
[4] Sendurai A, Wenjun G, Alicia Y, Mary B, Ferenc R, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. Volume 133, Issue 4, 704-715.
[5] Hart S, Novotny V, Williams M, Cheong A. VS-5584, a novel and highly selective pi3k/mtor kinase inhibitor for the treatment of cancer. Mol Cancer Ther; 12(2); 151–61.
[6] Naoko T, Pamela J. Ronald Q. Warren S. Percy I. Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nature Reviews Clinical Oncology. 97-106. doi:10.1038/nrclinonc.
[7] Xing F, William M, Leila K, et al. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumours. Cancer Res 2006;66:7445-7452.
[8] Daniel D. Patricia M. LoRusso, D. Charles M, et al. Inhibition of the Hedgehog pathway in advanced basal-cell carcinoma. N Engl J Med. 2009;361:1164-72.
[9] Shelton CC, Zhu L,et al. Modulation of gamma-secretase specificity using small molecule allosteric inhibitors. Proc Natl Acad Sci. 2009; 106: 20228-20233.

[10] Wang Y. Effects of salinomycin on cancer stem cell in human lung adenocarcinoma A549 cells. Med Chem 2011; 7: 106-111.
[11] Fong D, Yeh A, Naftalovich R, Choi TH . Chan MM. Curcumin inhibits the side population (SP) phenotype of the rat C6 glioma cell line: towards targeting of cancer stem cells with phytochemicals. Cancer Lett 2010; 293: 65-72.
[12] Bergstein, I. Methods of cancer therapy targeted against a cancer stem line. US8038998 (2011).
[13] Takebe N, Harris PJ, Warren RQ, Ivy SP. Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nat Rev Clin Oncol 2011; 8:97-106.
[14] Welte Y, Adjaye J, Lehrach HR, Regenbrecht CR. Cancer stem cells in solid tumours: Elusive or illusive? Cell Commun Signal 2010; 8:6.
[15] Sekulic A, Migden MR, Oro AE, Dirix L, Lewis KD, Hainsworth JD, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. N Engl J Med 2012; 366: 2171-9.
[16] Hassounah NB, Bunch TA, McDermott KM. Molecular pathways: The role of primary cilia in cancer progression and therapeutics with a focus on Hedgehog signaling. Clin Cancer Res 2012; 18:2429-35.
[17] Shannon M. Stefanie L. Gregory A. Katherine W, et al. Targeting Notch, a key pathway for ovarian cancer stem cells, sensitizes tumours to platinum therapy.PNAS plus 2012.
[18] Rute L, Katia A. Paulo J,et al. Mitochondria in Cancer Stem Cells: A target for therapy. Recent patents on endocrine, metabolic & immune drug discovery: 2013 Vol. 7 (2).
[19] Reya T, Morrison SJ, Clarke MF,Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001; 414: 105-111.
[20] Dean M, Fojo T and Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005; 5: 275-284.
[21] Jones RJ, Matsui WH , Smith BD. Cancer stem cells: are we missing the target? J Natl Cancer Inst 2004; 96: 583-585.
[22] Moltzahn FR, Volkmer JP, Rottke D and Ackermann R. "Cancer stem cells"-lessons from Hercules to fight the Hydra. Urol Oncol 2008; 26: 581-589.
[23] Visvader JE and Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 2008; 8: 755-768.
[24] Park CY, Tseng D , Weissman IL. Cancer stem cell-directed therapies: recent data from the laboratory and clinic. Mol Ther 2009; 17: 219-230.
[25] Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA , Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994; 367: 645- 648.
[26] Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumourigenic breast cancer cells. Proc Natl Acad Sci U S A 2003; 100: 3983-3988.
[27] Hemmati HD, Nakano I, Lazareff JA, Masterman Smith M, Geschwind DH, Bronner-Fraser M , Kornblum HI. Cancerous stem cells can arise from pediatric brain tumours. Proc Natl Acad Sci U S A 2003; 100: 15178-15183.
[28] O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immune deficient mice. Nature 2007; 445: 106-110.