CANCER STEM CELL BIOLOGY and CANCER VACCINES
(12)

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Therapies Targeting Cancer Stem Cells

- CSC therapy indicates cancer therapy by targeting the CSC population using different models.

- CSCs therapy is gaining much attention from the researchers because of its ability, to target, CSCs, which are responsible for initiation, progression and metastasis of cancer, unlike non CSCs cancer cells.

- Also, conventional cancer therapies are inefficient, in contrast to, CSCs therapy because the conventional cancer therapy involving chemo and radiotherapy often are unable to kill CSCs, thereby resulting in multiple malignancies, and are also toxic to the healthy tissues.

The Approaches to Target Cancer Stem Cells

1. Signaling pathway
2. Targeting cancer stem cell markers (cell surface markers)
3. Targeting drug detoxifying enzymes and drug efflux pumps present in CSCs
4. Targeting CSC niche and quiescent state
5. Targeting CSCs molecules using micro RNA
6. Inducing CSCs apoptosis and differentiation
7. CAF targeted therapeutics
8. Therapy against plasticity of CSCs
9. Targeting epigenetic regulators of CSCs
10. CSC-directed immunotherapy

1. Signaling pathway

- Common signaling pathways shared between normal stem cells and CSCs like Hedgehog (Hh), Notch, Wnt/b-catenin, Bcl-2, Bmi-1, etc. undergo aberrant activation or dysregulation to give rise to CSCs.
- Accordingly, targeting these signaling pathways by using various formulations of inhibitors of these signaling pathways as anticancer drugs are the answer to target the CSCs.
- One downside of using inhibitors of signaling pathways is the adverse effect of these inhibitors on the normal stem cells; hence, anticancer drug formulations involving signaling inhibitors should be made in combination with other CSC-targeting therapies to improve their specificity.
- Targeting specific factors that contribute to CSCs self-renewal and CSC proliferation by neutralizing those factors intra cellularly or affecting different parts of the Hh, TGF-β, and Wnt pathway in several types of malignances is another approach to control CSCs.
- Glasdegib is a small molecule which inhibits sonic hedgehog receptor smoothened and was recently approved by FDA in combination with low-dose cytarabine for newly diagnosed acute myeloid leukemia in patients with co-morbidities.

1. Signaling pathway

- **Focal adhesion kinase (FAK) inhibitors:** Regulated by OCT-3/4 and NANOG, FAK plays an important role in CSC self-renewal and tumor progression. There are more than 40 clinical trials evaluating the clinical safety or efficacy of FAK inhibitors.

- **Wnt/β-catenin inhibitors:** Numerous clinical trials have been initiated to evaluate the safety and/or efficacy of various molecules targeting the Wnt/β-catenin pathway in cancer cells. In general, these drugs have shown limited activity as single agents. However, in combination with chemotherapy and other compounds, significant response rates have been reported.

- **Notch inhibitors:** Monoclonal antibodies that can alter notch ligand-receptor binding and gamma-secretase inhibitors, which can block downstream signaling, are both in clinical development. Presently, there are more than 100 gamma-secretase inhibitors, and almost 50 clinical trials have been initiated to evaluate their clinical safety and efficacy. Early trial results indicate that the inhibitors are generally safe but associated with dose-limiting toxicities, predominantly of the gastrointestinal tract.

- **Hedgehog pathway inhibitors (HHi):** is essential for maintaining a stem-like state and is therefore exploited by CSC via epigenetic regulation and microenvironment activation. Indeed, a combination of the HHi Daurismo plus low-dose HDAC inhibitor cytarabine vs. cytarabine alone resulted in a doubling of overall survival in elderly patients with acute myeloid leukemia, resulting in FDA approval. There are currently more than 70 phase I-IV clinical trials involving inhibitors of the Hedgehog signaling pathway aimed at eradicating bulk tumors and CSC populations.

2. Targeting cancer stem cell surface markers

- Even though CSCs from different tissues have been characterized according to their phenotypic differences, there is still no definitive marker that only targets a specific population of CSCs.

- Currently, the identification and isolation of CSCs continue to be a challenge for therapeutic development; however, certain markers have been shown to be highly expressed among several different CSCs.

- In CSCs, a combination of several markers could be the best approach for specific targeting of CSCs; for example, in breast cancer, the most highly expressed CSC markers include CD133, CD44, and aldehyde dehydrogenase (ALDH).
  - ✓ **CD133** (prominin-1) is expressed on CSCs in multiple tumors, and high expression of this marker has been associated with poor cancer prognosis, making this molecule a good target candidate.
  - ✓ **CD44**, a glycosylated type-1 transmembrane glycoprotein, is involved in cell to cell interactions, cell proliferation and cell migration, among the most widely used CSC markers, it is associated with increased potential for tumor initiation and progression.

Tumor type	Markers
Leukemia	**CD34$^+$** [191, 196–198]; **CD38$^-$** [191, 197, 198]; **CD47$^+$** [191]; CCL-1 [191]; **CD96$^+$** [36, 191]; **CD90$^-$** [198]; **CD117$^-$** [198]; **CD133$^+$** [199]; **CD123$^+$** [191, 198]
Breast	**CD34$^+$** [200]; **CD24^{low}** [36, 48, 191, 197]; **ALDH1$^+$** [198]; **CD29$^+$** [201]; **Bmi-1$^+$** [202]; **CD133$^+$** [48]; **ESA$^+$** [191, 198]; **CD59$^+$** [36]
Pancreatic	**ESA$^+$** [48, 191]; **CD24$^+$** [48, 191, 198]; **CD44$^+$** [48, 191, 198]; **CD133$^+$** [36, 48, 198]
Lung	**CD44$^+$** [48, 191]; **CD133$^+$** [48, 191, 198, 203]; **CD59$^+$** [36]; **CD56$^+$** [203]
Liver	**ESA$^+$** [191]; **CD133$^+$** [48, 191, 198]; **CD90$^+$** [191, 198]; **CD44$^+$** [48, 191]; **CD176$^+$** [48]
Gastric	**CD133$^+$** [204]; **CD54$^+$** [48]; **CD44$^+$** [48, 191]
Colorectal	**ESA$^+$** [191]; **CD133$^+$** [36, 48, 191, 198, 205]; **CD166$^+$** [48, 191, 198]; **CD44$^+$** [48, 191, 198]; **CD24$^+$** [48, 191, 198]
Prostate	**Integrin α2β1** [48]; **CD44$^+$** [48, 198]; **CD133$^+$** [48, 198]; **Bmi-1** [206]
Melanoma	**CD34$^+$/CD31$^+$** [200]; **CD20$^+$** [198]; **CD44$^+$** [207]
Ovarian	**CD44$^+$** [48, 197, 208]; **CD117$^+$** [48, 197, 208]; **CD133$^+$** [48, 208]; **CD24$^+$** [48]

Rossi et al., 2020

2. Targeting cancer stem cell surface markers

- Both CD133, CD44 and CD24 have been identified on CSCs in breast, brain, colon, lung, prostate, liver, and gastric cancers, which makes these markers a potential target for neutralizing antibodies, antibody-mediated cancer stem cell therapies, or by engineering exosomes expressing these markers for drug delivery.

- Certain cytotoxic drugs, short hairpin RNA molecules, antibodies, which primarily target the cell surface markers of CSCs, can prove to be useful methods to target CSCs.

CD133: is involved in WNT/β-catenin signaling and is capable of regulating cell differentiation. Currently there exist a number of CD133-targeting antibodies. Most of these agents are still at the preclinical stage.

- Down regulation of CD133+ CSCs using short hairpin RNA in human metastatic melanoma had resulted in slower cell growth, reduced cell motility and decreased ability to form spheroids and reduced capacity of metastasis.

2. Targeting cancer stem cell surface markers

- **CD44:** is a transmembrane glycoprotein and expressed by many tumors. CD44 targeting therapies have shown efficacy in preclinical studies. CD44 antibody selectively kill CSCs in head and neck squamous cell carcinoma and has been shown to facilitate cellular uptake of doxorubicin, inducing chemo-sensitization. The first-in-human phase I clinical trial of an anti-CD44 monoclonal antibody (RG7356) for CD44-expressing local advanced or metastatic tumors was recently completed. This trial exhibited safety and efficacy.

- **CD24:** is expressed on the surface of cells, constitutes a prevalent CSC marker, has roles in cell signaling and has recently been demonstrated to promote tumor immune evasion. Preclinical studies with anti-CD24 antibody treatment has impeded tumor growth in hepatocellular carcinoma, colorectal and pancreatic adenocarcinoma, and reduced CSC populations. CD24 has been shown to be a novel *'don't eat me'* signal protein, most abundantly expressed in metastatic ovarian cancer and triple-negative breast cancer. CD24 allows tumor cells to evade phagocytosis. Antibodies targeting CD24 induces phagocytosis and obrogation of tumor growth.

3. Targeting drug detoxifying enzymes and drug efflux pumps present in CSCs

- Certain CSCs are rich in drug detoxifying enzymes like ALDH1.

- Studies have reported the inhibition of ALDH1 activity in breast CSCs using specific ALDH inhibitor diethylamino-benzaldehyde (DEAB) or all-trans retinoic acid (ATRA), which resulted in the reduced aggressiveness, and hence increased sensitivity of breast CSCs to chemotherapeutic drugs.

- Furthermore, ABC drug transporter proteins commonly shared between normal, and CSCs are efflux pumps that protect the cells from xenotoxins confer multiple drug resistance to the CSCs.

- Sensitivity of CSCs to chemo and radiotherapy was reportedly increased by targeting ABC drug transporter proteins via pharmacological drug molecules *in vitro* and *in vivo* in lung cancer cells.

4. Targeting CSC niche and quiescent state

- The CSC microenvironment is crucial for cancer initiation and tumor growth.

- It is not novel to try to target factors that potentiate tumor growth and dissemination as a therapeutic strategy; however, the problem is that these CSCs niches are enclosed and provide limited accessibility for most therapeutic antibodies or neutralizing cytokines.

- The use of exosomes in cancer therapy has gained more attention lately. Exosomes are small intracellular membrane-based vesicles involved in the delivery by endocytosis or membrane fusion of molecules, antibodies, or drugs. Exosomes expressing unique surface markers could be used to deliver drugs or molecules to a specific CSCs population to kill these cells.

4. Targeting CSC niche and quiescent state

- CSC microenvironment promote an immunosuppressive state and do not allow for a proper immune response, suggesting that one alternative approach to target this microenvironment is to blocking the action of myeloid suppressor cells action and targeting cellular receptors involved in immunomodulation, like PD1/PDL-1.
- For example, targeting VEGF-A with the humanized recombinant monoclonal antibody, bevacizumab. This drug inhibits the angiogenesis process by slowing down the growth of new blood vessels and disrupting the CSCs niche.
- It has already been shown in a glioblastoma murine model that treatment with bevacizumab depletes vasculature to the CSC microenvironment and drastically reduces the number of glioblastoma stem cells.
- Fresolimumab, a monoclonal antibody that binds to all isoforms of TGFβ and thus modulates tumor microenvironment, has been tested in clinics in combination with focal irradiation for the treatment of metastatic breast cancers.

5. Targeting CSCs molecules using micro RNA

- This has been shown by the use of exosomes to deliver specific miRNAs that block or arrest key CSCs signaling pathway.
- SSCs express a unique set of miRNAs that maintain self-renewal, promote differentiation, and maturation.
- However, in CSCs, these miRNAs are deregulated. For example, the overexpression of miRNA-124 and miRNA-137 in human glioblastoma derived cancer stem cells resulted in the loss of selfrenewal and oncogenic capacity.
- It has shown that blockage of miR-21 reverses EMT and CSC phenotype, suggesting that miRNA-21 has a role in CSC maintenance and this molecule could be targeted in future therapies.
- No clinical trials utilizing such strategy are currently registered at clinicaltrials.gov but a number of promising pre-clinical studies have been published.

6. Inducing CSCs apoptosis and differentiation

- CSCs produce anti-apoptotic proteins, which confer chemo and radio resistance to them.

- Interleukin-4 is one such anti-apoptotic protein reportedly produced by CD133+ colon carcinoma cells. Successful targeting of IL-4 using IL-4 neutralizing antibody and IL-4 antagonists have proven to increase the sensitivity of CD133+ colon carcinoma cells to chemotherapeutic drugs (oxaliplatin and 5-FU).

- Apart from the approach to kill CSCs, another approach is to inhibit the self-renewal of CSCs by inducing of terminal differentiation using differentiating agents like all-trans-retinoic acid and retinoids and HDAC inhibitors.

7. CAF (cancer-associated fibroblast) targeted therapeutics

- Fibroblast activation protein alpha (FAPα): is expressed in the CAFs of ~90% of all carcinomas.
- Substantial efforts have been made to target CAFs using FAPα, with disappointing single agent results.
- More recent trials have used FAPα inhibitors in combination with other agents; however, these, too, have shown limited clinical efficacy.
- Currently, there are three ongoing clinical trials evaluating the efficacy of the novel bispecific FAP-DR5 antibody RO6874813 as a single agent or in combination therapy.
- An alternative approach to deplete CAFs is under development which uses a FAPα vaccine. Pre-clinical data demonstrated that FAPα vaccines were able to suppresses tumor growth and metastasis in colon and breast cancer models. However, no clinical trials have tested these vaccines.

8. Therapy against plasticity of CSCs

- **Plasticity** is a process by which cancer cells gain the dynamic ability to switch from non-cancer stem cells to cancer stem cells and vice versa. Phenotypic plasticity is the ability of cells to differentiate into multiple lineages. In normal development, plasticity is highly regulated whereas cancer cells re-activate this dynamic ability for their own progression.

- Presently, several clinical strategies have been proposed that could be effective against plasticity of CSCs:

1. Therapeutic blocking or reversing de-differentiation of CSCs as they could be generated de novo by dedifferentiation of non- CSCs, which prevent cancer cells from becoming metastatic and developing drug-resistant phenotype.
2. Therapeutics could be beneficial to patients by neutralization factors for EMT.
3. Treatment can be targeted by blocking the signalling pathway used by EMT cells to evade, survive in the circulation, or resist therapy against cancer.

✓ Thus, it is critical to destroy cancer cells that undergo EMT block or reverse that process. Interestingly, various approaches have already been taken to fight with signalling pathways that induce EMT such as TGF-β, STAT3, miR-210.

8. Therapy against plasticity of CSCs

- Targeting metabolic plasticity of CSCs has become an emerging area to effective elimination of CSCs. To inhibit glycolysis in CSCs, glucose transporter and glycolytic enzymes such as GLUT1-4, hexokinase1-2, pyruvate kinase M2, and lactate dehydrogenase have been proposed as attractive targets.

- Moreover, mitochondrial metabolism could be a useful target to eliminate OXPHOS phenotype of CSC in numerous cancers.

- Another potential target is the inhibition of adaptive mechanism of CSC. Under hypoxia, glucose deprivation, and low pH, CSC rapidly transits their metabolism and this adaptive metabolic switch by CSCs plays a pivotal role in cancer metastasis or chemo-resistance.

There are several factors or enzymes involved in the metabolic adaptation of CSCs, which are sensitive to specific therapeutic actions. For example, HIF1-2 alpha is a key enzyme for metabolic adaptation in hypoxia and is associated with angiogenesis, metastasis, and cell survival.

9. Targeting epigenetic regulators of CSCs

- Histonedeacetylase inhibitors (e.g. suberoylanilide hydroxamic acid, abexinostat) have been shown to promote differentiation of breast cancer cells and diminish the number of CSCs within cancers.

- Moreover, a key feature of epigenetic mechanisms is their inherent reversibility, which helps cancer cells to gain cellular plasticity. This dependence of CSCs on epigenetic regulators offers an opportunity to target their self-renewal capacity.

10. CSC-directed immunotherapy

- Some novel anti-CSC immunotherapeutic approaches, such as immunologic checkpoint blocking or CAR-T cell therapies, have been developed.

- Some drugs that target the immune checkpoint receptors CTLA-4, PD-1 (nivolumab, pembrolizumab, and cemiplimab) and PD-L1 (avelumab, durvalumab, and atezolizumab) have also been used in clinical trials.

- A CTLA-4 antibody (ipilimumab), is approved by the FDA, and initial clinical results showed good effectiveness in patients with metastatic melanoma.

CSC-directed immunotherapy in ongoing clinical trials

Trial description	Condition	Sample size	Phase	NCT Number	Current status
CD19 CAR-T	B cell leukemia and lymphoma	II	80	NCT03398967	Recruiting
CD123 CAR-T	$CD122^{+}$ myeloid malignancies	II	45	NCT02937103	Recruiting
CD22 CAR-T	Recurrent or refractory B cell malignancy	I/II	45	NCT02794961	Unknown
CD22 CAR-T	B-ALL	I	15	NCT02650414	Recruiting
CD33 CAR-T	Myeloid malignancies	I/II	45	NCT02958397	Recruiting
CD33 CAR-T	$CD32^{+}$ acute myeloid leukemia	I	11	NCT03126864	Active, not recruiting
CD38 CAR-T	B-ALL	II	80	NCT03754764	Recruiting
CD138 CAR-T	Multiple myeloma	II	10	NCT03196414	Recruiting
MUC1 CAR-T/PD-1 KO	Advanced esophageal cancer	I/II	20	NCT03706326	Recruiting
EGFR IL-12 CAR-T	Metastatic colorectal cancer	I	20	NCT03542799	Not yet recruiting
MESO CAR-T	Refractory–relapsed ovarian cancer	I/II	20	NCT03916679	Recruiting
MESO-19 CAR-T	Metastatic pancreatic cancer	I	4	NCT02465983	Completed
LeY CAR-T	Myeloid malignancies	I/II	445	NCT02958384	Recruiting
MOv19-BBz CAR -T	Recurrent high-grade serous ovarian cancer	I	18	NCT03585764	Recruiting
LeY CAR-T	Advanced cancer	I	30	NCT03851146	Recruiting
EpCAM CAR-T	Recurrent breast cancer	I	30	NCT02915445	Recruiting
BCMA CAR-T	Multiple myeloma	II	80	NCT03767751	Recruiting

Yang et al., 2020

Conclusion

- ✓We can conclude that CSCs are a small population of cancer cells that have self-renewal capacity and differentiation potential, thereby conferring tumor relapse, metastasis, heterogeneity, multidrug resistance and radiation resistance.
- ✓Several pluripotent transcription factors, including Oct4, Sox2, Nanog, KLF4, and MYC and some intracellular signaling pathways, including Wnt, NF-κB, Notch, Hh, JAK-STAT, PI3K/AKT/mTOR, TGF/Smad, and PPAR, as well as extracellular factors, including vascular niches, hypoxia, TAM, CAF, cancer-associated MSCs, the ECM, and exosomes, are essential regulators of CSCs.
- ✓Drugs, vaccines, antibodies, and CAR-T cells targeting these pathways have also been developed to target CSCs.
- ✓Importantly, many clinical trials on CSCs have also been performed and show a promising future for cancer therapy.

Conclusion

- ✓It is important to note that, even though we are still early in the course of CSC-targeting therapeutic development, we are beginning to see important clinical successes that increase hope that CSC targeting will indeed improve patient outcomes.

- ✓Positive phase II trials with CSC targeting drugs metformin and disulfiram, showing significant improvements in patient overall survival, should encourage translational scientists to re-double their efforts at targeting CSCs clinically.

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