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Recent Advances in the Understanding of CSCs and Treatments Targeting Them

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ABSTRACT

This study overviews the biology of the cancer stem cell (CSC) and current progress made in treatments targeting them. CSCs help cancer to resist current mainstream cancer treatments and allow cancer to recur even after it is considered cured. CSCs are cancer cells that are both low in number and divide slowly, or even stay quiescent (not multiplying). This allows them to avoid being targeted by chemotherapy and other therapies that are created to target the rapidly dividing cancer cells. To combat the CSCs researchers have looked at biomarkers that are specific to CSCs, however it is difficult. CSCs have many biomarkers in common with other cancer cells or with non-cancerous counterparts. In addition, biomarkers that are present in one type of cancer may not be present elsewhere. Despite this, many CSC biomarkers have been identified as possible means to target them. Other biomarkers, like CD26, have been identified as early signs of an aggressive cancer and can serve as a warning, allowing time for preparation. Many more currently ongoing studies are also looking for even more biomarkers that can be used to target CSCs effectively. Other studies look at possible ways to use those biomarkers against cancer, using methods such as immunotherapy. While no definitive method for combating CSCs currently exists as of now the subject is showing great potential as a method to help treat cancer.

Introduction

Cancer is a category of diseases that encompasses many types of unchecked dividing cells in any part of the body. It is the number two cause of death in the U.S. and accounts for 10 million or about 1 in 6 deaths globally (World, 2022). Cancer is one of the most feared diseases in the world with millions of cases appearing yearly (World, 2022). Cancer has no known cure as of today, but there are many treatments for it. Cancer is difficult to treat because in order to treat it, you need medicine which can identify which cells are cancerous and then attack only those cells. However, the problem with that is cancer originates from self-cells and, except for some mutations, is almost identical to their normal counterparts. This is why some treatments, such as chemotherapy, cause the hair and bone marrow cells to be targeted as well, since the treatments attack all rapidly dividing cells, not just cancerous ones. More recently, another blockade to the development of the cure for cancer has revealed itself in the form of Cancer Stem Cells (CSC). CSCs are cells which have the potential to turn into many other types of cells. If cancers have their own pool of stem cells, then simply attacking the cancer is not enough since the stem cells can simply regenerate the cancer indefinitely. It is difficult for current cancer treatments to affect CSCs because they do not divide as rapidly as other cancer cells, therefore allowing them to survive by avoiding detection. Some newly developed therapies however have found some potential ways to deal with CSCs. Researchers are looking to target biomarkers in CSCs and hope to find a way to detect and target these cells without harming the normal human cells.

CSCs

According to current theory, CSCs are cells that can infinitely regenerate themselves and replace other cells (Figure 1). They are relatively slower at dividing or are even completely quiescent (dormant), which allows them to evade detection from common treatments such as chemotherapy. Another key trait about them is that they live in their own niches, places that are specialized for them (Plaks et al., 2015).

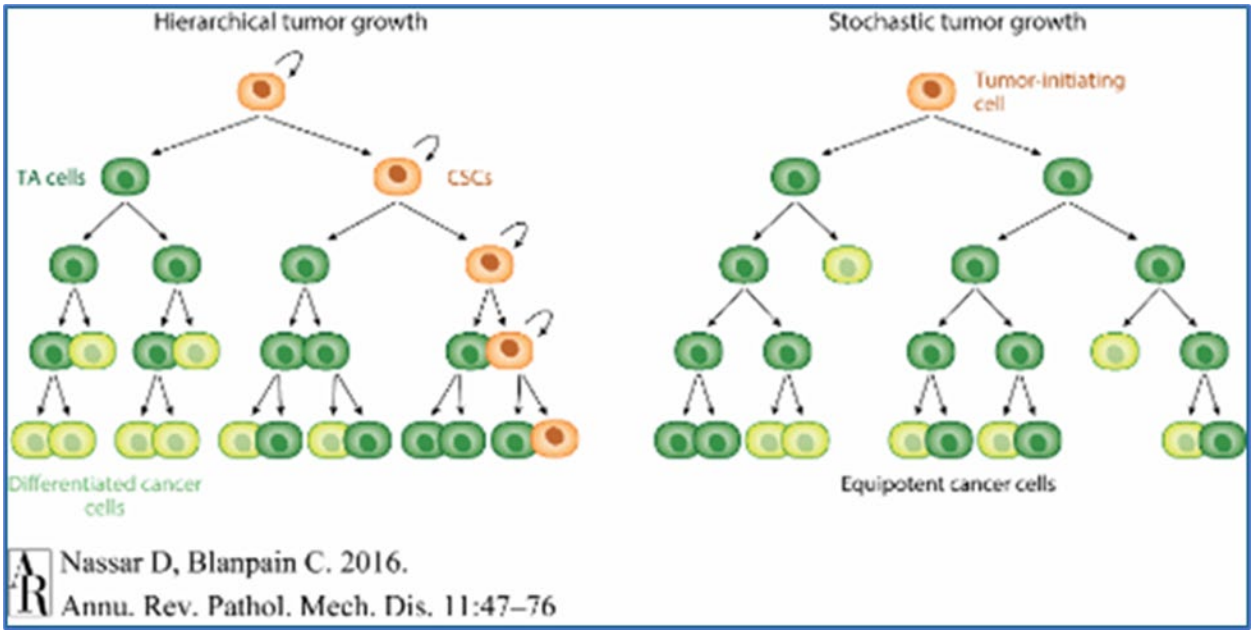

Figure 1. CSC Models. Source: Nassar & Cédric Blanpain, 2016. Description: Two different models for how CSCs work., In the Hierarchical model, CSCs are shown to be infinitely regenerating and turning into other cells. In the Stochastic model, there is no such stem cell and the cancer is simply growing and spreading. The Hierarchical model, the one on the left shows the newer hypothesis, while the one on the right shows what was originally thought to be correct.

Subsequent understanding of CSCs showed that they are even more advanced than shown in the diagram above. In fact, the CSCs can choose how many CSCs they want to produce from cell division based on the circumstances and how much space is available in their niche (Batlle & Clevers, 2017).

CSCs have been found in many different types of cancers (Barbato et al., 2019) (Erina Vlashi & Pajonk, 2015). These stem cells were identified when recent research studies observed highly differing types of cell clones in cancers which suggested that the hierarchical tumor growth model was correct. In addition, it was found that specific clones correlated with an increase in the chances of a tumor recurrence (Nassar & Cédric Blanpain, 2016).

The researchers then looked for the most recent type of cancer cell that all other cells branched out from, the newest common ancestor. It was found that that specific type of cell had actually been in the tumor since the very start of the tumor growth and rather than multiplying it kept a stable number and instead passively mutated itself. These mutations were the ones that then multiplied in number and increased the size of the tumor, not the original cells that they stemmed from. The original cells in fact did not even grow in proportion to the size of the tumor, but instead simply created a better environment for the other cancer cells that it spawned. This makes the cells 'tumor initiating cells', meaning that they can create tumors by themselves as long as at least one of them survives (Biserova et al., 2021). This once again closely follows the model with CSCs. The cells also were believed to show only

unidirectional progression, meaning the cells only mutated away from the origin cells, never closer towards it (Walcher et al., 2020). Further research also found more parallels between these cells and normal human stem cells (Nassar & Cédric Blanpain, 2016).

CSC's Role in Cancer Progression and Therapy Resistance

Now that CSCs existence and their basic roles in cancer have been established, it is time to delve a little deeper. It is now time to explore why exactly it is that CSCs are a problem and how they inhibit cancer therapy from working as it should.

CSCs have a few traits that make them stand out from others, they divide slowly, are low in number, are resistant to treatment, and correlate with recurrence of tumors even after the cancer is considered cured (Biserova et al., 2021). CSCs' main method of defending itself from common therapies such as chemotherapy is its slow division, the cells were even shown to divide while chemotherapy was ongoing without being attacked (Nassar & Cédric Blanpain, 2016). This allows for tumors to relapse, even once other cancer cells have been killed by chemotherapy meaning that most current methods for dealing with cancer are ineffective since they focus only on rapidly dividing cells (Barbato et al., 2019). In fact, some types of cancer, such as, "Hepatocellular carcinoma (HCC), the most common liver cancer" can have recurrence rates as high as 70%. This shockingly high number is attributed to the common therapies lack of attention to CSCs which regenerate the cancer once the therapy ends (Sun et al., 2016).

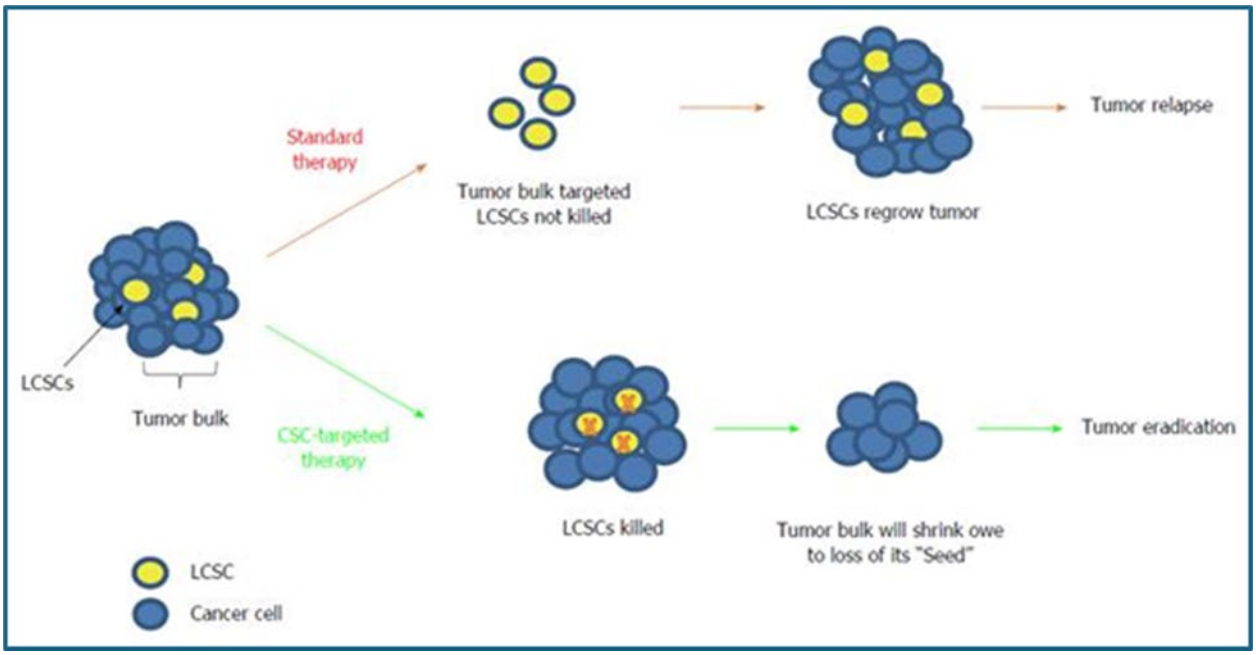

Figure 2. Model for Therapy Outcomes Based on Therapy's Target. Source: Sun et al., 2016. Description: This is an oversimplified diagram of the prognosis associated with different types of cancer therapy. It shows how when CSCs are not erased, they can regrow the entire tumor, causing once supposedly cured cancers to relapse over and over again.

CSCs can be found in any cancer, not only in cancers that originate from normal stem cells. This means that the stem cells are created by the cancer itself (Quaglino et al., 2020). Therefore, new therapies that also attack slower diving cells need to be produced in order to battle all types of cancers (Barbato et al., 2019). Even therapies like

immunotherapy, which utilizes the body's immune system to fight against cancer, are also reduced in effectiveness by CSCs' ability to produce immunosuppressive cytokines, which inhibit NK and T cells (Quaglino et al., 2020).

Biomarkers of CSCs for Diagnostics and Treatments

Scientists have made progress on finding ways to develop these new therapies to battle against CSCs. They first need a way to track down the CSC, so they find biomarkers that can be used to identify them, for example, breast CSCs are identified with specific antibodies for CD44". These biomarkers can tell antibodies or other treatment methods what and where to attack, allowing for specific targeting of CSCs without harming any of the body's normal cells. However, this is not always the case as many biomarkers are also present in normal cells as well, so only a few biomarkers will actually work (Barbato et al., 2019). The biomarker that is used also needs to be a biomarker that is essential for the CSCs' very existence, for example the instructions for the CSC to divide slower than the other cancer cells. This will help protect against the CSCs evolving immunoresistance against the therapies through natural selection.

The same marker may not be usable for all CSCs because many biomarkers are specific to the tissue from where the cancer originated, meaning that each type of cancer would need its own biomarker to be identified (Barbato et al., 2019). In addition, even among cancers of the same type there are different markers depending on the patient or lesion from where the sample was obtained.

One example of this for each of the last two ideas mentioned are brain tumors which have the same markers as their origin cell, and intestinal tumors, where a marker called Dclk1, that is only present in CSCs, not normal stem cells, was found. (Nassar & Cédric Blanpain, 2016). This also implies that the CSCs could be developed without needing any assistance or DNA from human stem cells. In some other types of cancer, the stem cell might even lose their biomarkers making them more difficult to track and also showing that biomarkers are not failproof. It is also important to note that if a biomarker is found that does not mean it will appear in all cancers, not even in cancers of the same type (Erina Vlashi & Pajonk, 2015).

Biomarkers still can reveal much information, for example the biomarker ALDH1 was correlated to a poor prognosis, or outcome for breast cancer. It was also used to show what tumors could form from as few as twenty CSCs (Erina Vlashi & Pajonk, 2015).

Another biomarker, CD26 was found as a marker for aggressive tumors in hematological malignancies, however, the extent of the cancers for which it is present is still to be seen (Khaled, 2024). Other markers, 7-miRNAs10-mRNA, were used to accurately identify early on which patients' lung adenocarcinoma would become aggressive once more developed (Bianchi, 2024). Another marker that was found to be closely correlated to more deadly cancer for patients with lung adenocarcinoma was ALDH2. It was shown to be an effective prognostic marker and a good way to tell what kind of therapy would be needed (Tran et al., 2023).

Some biomarkers actually correlate to greater therapy resistance even, and it is believed that these markers correlate to CSCs. Other biomarkers relate to things like therapy response speed and the chance of a tumor recurrence. Many biomarkers also have contradicting results based on who conducted the study and therefore may not be very reliable (Walcher et al., 2020).

Treatments can also be designed to only attack when multiple biomarkers are found, allowing for greater accuracy than possible with just a single marker.

Overall biomarkers are showing promise as a possible way to overcome CSCs, but more research still needs to be done on the biomarkers that have been identified thus far. It is also possible that science will reveal another biomarker that is more useful in this pursuit in the future and then the focus of research may shift.

Current Therapies

There are many studies that look into CSCs and how to target them using biomarkers, among the early studies experiments among mice are common. For example, they were used in early studies to confirm the existence of CSCs (Batlle & Clevers, 2017), and also used to demonstrate that CSC immunotargeting is possible (Quaglino et al., 2020). The studies also showed that since the trials went well, targeting of biomarkers in CSCs might even be better than other cancer cells (Quaglino et al., 2020).

Some cancers, such as brain cancer currently do not have an effective method for curing the disease as its stem cells are both radiotherapy and chemotherapy resistant, targeting their stem cells should allow for other treatment methods to become effective, solving this issue (Granda, 2024).

The only problems involved in the targeting of biomarkers using immunotherapy are not just related to identifying biological markers, however. Some studies have been conducted to find how much of a vaccine a person can take. Cancer vaccines work just like any vaccine, putting in weaker cells with the same biomarkers to train the immune system. If a vaccine is too strong it will overload the body's immune system and may lead to autoimmune responses. On the other hand if it is too weak it will have a lesser effect on the body's ability to fight against the disease. Therefore, studies attempt to find a balance using trial and error and slowly increasing dosages to see where the fine line between the two sides are (University of Minnesota, 2024).

Table 1. Ongoing clinical studies identifying or targeting CRCs in various cancer types. Description: These trials are current studies that are looking into CSCs and their focuses are either diagnostic type testing or testing vaccines strength and effectiveness. This shows that while much research is being done in this field, there are still many things to explore. Many different biomarkers are being looked at to see if they are a valid way of targeting CSCs. Some therapies, like those involving dendritic cells do not even require the bi-omarkers to be identified by humans, and will identify biomarkers on their own. This field of study is also current-ly being explored.

Trail #	Type of Cancer	Biomarker Targeted	Type of Intervention	Institute/ Author
NCT04312607	Philadelphia chromosome positive MPN (CML) and Philadelphia chromosome negative MPN (PV, ET, PMF)	CD26 positive stem cells	Diagnostic Test: BCR-ABL gene PCR	Safaa AA Khaled, Assiut University
NCT06409416	Non-small Cell Lung Cancer	7-miRNAs/10-mRNAs signatures	Analysis of diagnostic biomarkers	Fabrizio Bianchi, Casa Sollievo della Sofferenza IRCCS
NCT06348693	Tumors of the central nervous system	Sphingosine-1-phosphate (S1P)	Cell isolation from tumor biopsies and biomarker investigation	Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico

NCT01171469	Progressive Malignant Brain Tumor	Brain Tumor Stem Cell Antigens	Dendritic Cells and Imiquimod	Masonic Cancer Center, University of Minnesota
NCT00846456	Glioblastomas	Tumor Stem Cell Derived mRNA-Transfected Dendritic Cells	Vaccine with mRNA from tumor stem cells	Oslo University Hospital

Although searching for biomarkers to eliminate CSCs is a popular method as of now, other strategies exist as well. Other strategies that have seen some success include boosting CSC's division rate to make it susceptible to chemotherapy. Another does the total opposite, instead attempting to stop the CSCs from dividing at all, thus rendering them harmless (Batlle & Clevers, 2017).

Conclusion

CSCs divide slowly, evading detection and allowing them to reform cancers even after they are considered cured. Targeting CSC biomarkers seems to be an exciting prospect that holds potential to help treat cancer and stop this recurrence. If proper research and effort is put into this field of study, then it is likely to become an essential step on the path to finding a cure to cancer. While no perfect method to target all CSCs currently exists. There are many experiments with vaccines and other treatment methods that have shown some success, but also have seen setbacks. Since biomarkers are inconsistent, it may be possible to create multiple medicines, each targeting a different set of biomarkers in order to cover most if not all types of CSCs. Future research should focus on finding the biomarkers that are CSC specific, allowing therapies to directly attack only CSCs.

Limitations

Biomarkers are not yet completely understood, they could mean little in regards to CSCs since correlation does not equal causation. All new info should be taken with a grain of salt as newer info may emerge with the advancement in technology.

Normal cancer cells sometimes revert back into CSCs, making the models not completely accurate, this is referred to as tumor cell plasticity. In fact some models predict that every single cancer cell has the potential to become a CSC (Erina Vlashi & Pajonk, 2015). This also explains why cancer seems to have unlimited growth potential since a limited number of stem cells limits the maximum possible growth speed.

Targeting only CSCs will not actually cause a tumor to shrink in size at all, both regular and CSCs need to be targeted in order to defeat the cancer. Therefore, it is beneficial to pick a biomarker present in both types of cancer cells (Quaglino et al., 2020).

A study on breast cancer found that their immunotherapy treatment was only effective on relatively newer cancers, whereas more developed ones were better able to withstand the treatment method (Quaglino et al., 2020).

This is not an exhaustive review, there are more biomarkers than just these that have been covered and there are many more treatments that were not looked into here. It is possible for another study to have already found a way to effectively target CSCs, albeit with some flaws in that method as well. The study and its research would still be relatively new, and all aspects would not have been looked at yet.

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