Cancer stem cells, do they exist or not?

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Abstract

Cancer stem cells are considered to be a small subpopulation of cancer which are thought to be responsible for the initiation and maintenance of cancer. These cancer stem cells are considered to be resistant to radiotherapy and chemotherapy and seem to be important in the relapse of cancer. However, there is a lot of controversy about this topic. Some aspects are difficult to prove and therefore it becomes uncertain if cancer stem cells exist or not. The cancer stem cell model states that there is a hierarchy present within tumors. A small subpopulation of cells should be the cancer stem cells which are on top of the hierarchy and have a self-renewal system like normal stem cells. The majority of the population are differentiated cells. These form the bulk of the tumor but have little to contribute to cancer progression. The problem with the cancer stem cell model is that it does not address the cell-of-origin. It does not show where the cancer stem cells come from and therefore if they are really stem cells. Also, this model is only tested in vitro or in mice and it is therefore not certain how and if this model works in patients and various types of cancer. To test the cancer stem cell model, it is of great importance to characterize and identify the cancer stem cells. This can be done using markers. This can be done by identifying and isolating cells based upon their cell surface expression of these markers. Some types of cancer are proven to have a small subpopulation of cells with different levels of markers than the majority of the cells in a tumor. Unfortunately all of the found markers are also present in other tissues and on non-tumorigenic cells. To prove the cancer stem cell model, a marker has to be found which is only present in cancer stem cells or at least another way has to be found to characterize cancer stem cells. To do so, an assay has to be developed which is highly specific, quantitative, and sufficiently sensitive. At the moment the best assay is serial transplantation in animal models. But when the field emerges simpler, more sophisticated and better assays will be developed. Given all this data, the conclusion is that it is too early to say if cancer stem cells or the cancer stem cell model are true. However, they seem to be possible to exist in at least a few types of cancer. When the field emerges and better ways are found to characterize and identify cancer stem cells, a better image can be formed about the cancer stem cells and the cancer stem cell model. For now, cancer stem cells remain a promising mystery.

Introduction

Recently, it was suggested that tumors contain a heterogenous population of cancer stem cells. They are thought to be responsible for the initiation and maintenance of cancer and have recently been characterized for several types of cancer as for example breast cancer (Nigam, 2013). These stem cells are considered to be resistant to radiotherapy and chemotherapy and are thought to be important for the relapse of cancer in patients. There

are two critical properties which are considered to be responsible for this: their capacity for self-renewal and their ability to differentiate into heterogeneous populations of cancer cells (B. Bao, Ahmad, Azmi, Ali, & Sarkar, 2013). These findings suggest that these cancer stem cells could play an important role as novel therapeutic targets for the treatment of cancer. An important part for the development of CSC-targeted therapies is the characterization and identification of potential cancer stem cells. It is suggested that this can be done by using markers. Certain markers seem to be enhanced in a small subpopulation of cancer. However, it seems to be that these markers are different for different types of cancer. These small populations of cells with enhanced markers indeed show an enhanced self-renewal of differentiation potential and seem to be more resistant to therapy (S. Bao et al., 2006).

There is also a lot of controversy about the topic of cancer stem cells. Some aspects are difficult to prove. It is, for example, uncertain if all cancers follow the cancer stem cell model or if only a few do. Also some cancer stem cell markers have been difficult to confirm and tumorigenic cell frequencies can sometimes change dramatically as a result of alterations of assay conditions (Magee, Piskounova, & Morrison, 2012). It is also uncertain if these cancer stem cells actually exist or that all cancer cells have the potency to behave like cancer stem cells. In that case it is possible that some cancer cells behave like cancer stem cells and switch with other cancer cells between a normal state and a stem cell state. If that is the case, it is impossible to target these cells as all cells could have the property to obtain cancer stem cell like characteristics (Reya, Morrison, Clarke, & Weissman, 2001).

Cancer stem cells could be an interesting target for novel therapies, if they exist. In this review, I will deal with various aspects of the cancer stem cell hypothesis because of the controversy about this topic. By doing so, I hope to answer the following questions: Do cancer stem cells exist and if so, how can we characterize and identify them? Also, what does this mean for novel therapies? My aim is to clarify a little bit more about the cancer stem cell hypothesis by answering these questions and hopefully to contribute to the debate if they are real or not.

The cancer stem cell model

The cancer stem cell model is already a couple of decades old (Hamburger & Salmon, 1977).It has been clear that some cancers can differentiate into cells that have limited proliferative potential despite the oncogenic mutations of their malignant progenitors. The presence of differentiated cells in a tumor residue after chemotherapy is a favorable factor as they stop dividing after some time. The presence of undifferentiated cells in a tumor residue after chemotherapy is not a favorable factor as they will not stop dividing. They will continue making new cells which will differentiate again. The presence of undifferentiated cells in a tumor residue predicts disease recurrence (Stenning et al., 1998).This suggests that undifferentiated cells are responsible for disease progression and tumor growth. They are therefore an interesting target for cancer therapy.

Cancers that follow the cancer stem cell model have different subpopulations of tumorigenic and non-tumorigenic cells. These cells are organized in a hierarchy in which a small population of cells have tumorigenic properties. These tumorigenic cells are the possible cancer stem cells. The possible cancer stem cells show self-renewal and are thought to be responsible for cancer progression and tumor growth. Differentiated cells or non-tumorigenic cells are phenotypically diverse and are daughter cells of the cancer stem cells. Just like the normal stem cell model. They can divide but will eventually die in contrary to the tumorigenic or cancer stem cells. The differentiated cells are thought to be the bulk of the tumor but have little to contribute to cancer progression (Dick, 2008; Reya et al., 2001; Shackleton, Quintana, Fearon, & Morrison, 2009). In this model, the cancer stem cells show self-renewal and stay the same like in the normal stem cell model as seen in figure 1. They also form other cells which will differentiate. These differentiated cells are diverse and form the largest part of the cancer cell population while cancer stem cells are thought to form a very small part of the population (Reya et al., 2001).

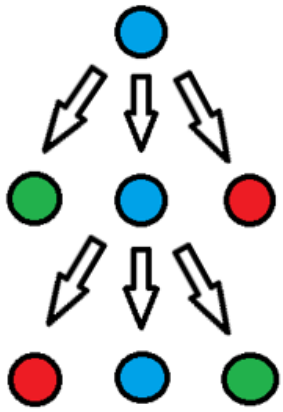

Figure 1: The cancer stem cell model. Cancer stem cells (blue) show self-renewal. They stay the same but also divide and form other cells (green and red). These cells are differentiated and phenotypically different. They will cause the bulk of a tumor and have little to contribute to cancer progression.

Flaws of the cancer stem cell model

However, despite all of these studies, the generalizability of the model is uncertain. Does it apply to all cancers or only to a few? Do all tumors of a certain type of cancer follow the cancer stem cell model or only in certain patients. There are differences among cancers in driver mutations. Also the cell-of-origin creates a problem. These questions complicate the testing of the model and could mean that observations of cells in a cancer in one patient may be true for cancers in other patients, but it may also not be true for cancers in other patients.

Some types of cancer can arise from normal stem cells by mutations that over activate certain mechanism such as the self-renewal mechanism. This includes cancers that are thought to follow the cancer stem cell model (Barker et al., 2009).Other cancers can arise from already differentiated cells as a result of mutations. These mutations activate, among other things, the self-renewal mechanism (Magee et al., 2012). Therefore, it does not mean that a cancer originated from normal stem cells even when it follows the cancer stem cell model as seen in

figure 2. Mutations in already differentiated cells can cause a tumor to arise with stem cells. Which means that they have to be able to re-install self-renewal. These stem cells originated from already differentiated cells. Thus, the cancer stem cell model does not address the cell-of-origin. Also, all of these findings are based on experimentally induced cancers. The cell-of-origin that spontaneously arises in cancers in most patients has not yet been identified. At least it is not identified with precision which makes it difficult to determine the way the cell-of-origin works within human cancers. Therefore it also makes it difficult to determine if the cancer stem cell model works the way it does in humans as it does in vitro. That means that treatment in patients cannot yet be used as if the cancer stem cell model is true.

Plasticity of cancer stem cells

Another idea states that cancer stem cells and differentiated cells can switch places as seen in figure 3(Magee et al., 2012). This plasticity could be of great interest in understanding cancer stem cells. Cancer stem cells form a small subpopulation but can become differentiated and therefore non-tumorigenic. A differentiated cell can become a cancer stem cell and therefore tumorigenic. This concept is plausible given the fact that restricted progenitors dedifferentiate into stem cells in certain normal tissues (Magee et al., 2012). For example, in mouse testis spermatogonial progenitors can dedifferentiate into spermatogonial stem cells (Barroca et al., 2009). However, this process has only been seen in normal tissues under restricted circumstances or low frequencies. Nonetheless, if it is possible in these cells, it could also be possible in tumors. This means that there is no established population of cancer stem cells. Therefore, there is also no real hierarchy. There are some stem cells, but any cell can switch back and forth so there is no established hierarchy. Thus, this population cannot be targeted. However, even though all cells could potentially become cancer stem cells, it is possible that at any given time point an established cancer stem cell population exists. Even though there is plasticity, there are always cancer stem cells present which may be possible to characterize and identify. It could be possible to distinguish between these cancer stem cells and the rest of the cancer cells as they might change and gain certain stem cell characteristics. This would also mean that at any given time point a hierarchy does exist as there is always a cancer stem cell population present. If the cancer stem cell population would be targeted and killed, other already differentiated cells, would change into cancer stem cells. This also means that the ability to distinguish non-tumorigenic cells from tumorigenic cells becomes impossible as even non-tumorigenic cells could be expected to form tumors. However, if this idea is true, it would be possible to target both populations and destroy the cancer cells that way. The problem is however, that these cells will show a great variance in phenotypes and are therefore hard to characterize and identify.

The cancer stem cell model does not effectively describe these types of cancers in which there are both tumorigenic and non-tumorigenic states that can reversibly interconvert. If cells in the non-tumorigenic state only convert to the tumorigenic state under certain circumstances or with low frequency and/or low efficiency, it could be possible to find markers to identify the population of cells which are tumorigenic and the cells that are non-tumorigenic. However, if the cells which are in the non-tumorigenic state can convert to the tumorigenic state with high efficiency and/or frequency, it could not be possible to identify tumorigenic

cells from non-tumorigenic cells. Which means that the cancer stem cell model would not be applicable in these types of cancer. In that case, new models might be necessary to describe this system.

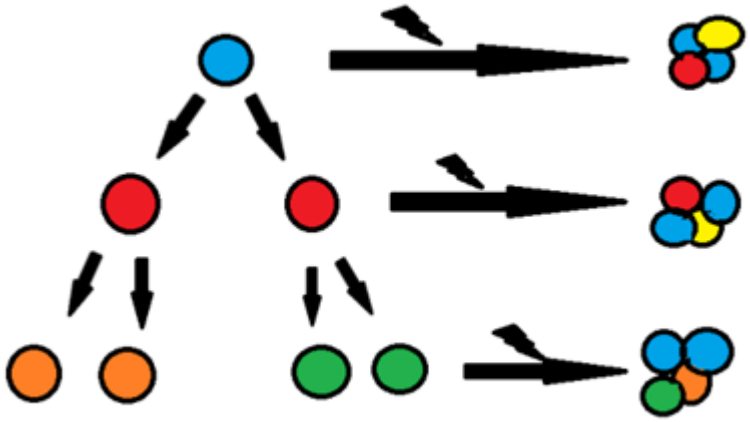

Figure 2: Cell-of-origin within the normal tissue hierarchy. Different cell types in a normal hierarchy. Stem cell (blue) on top of the hierarchy. Mutations cause a tumor with stem cells. Further differentiated cells (red) are differentiated from stem cells. Mutations cause a tumor with new stem cells. Fully differentiated cells (orange and green). Mutations cause a tumor with new stem cells.

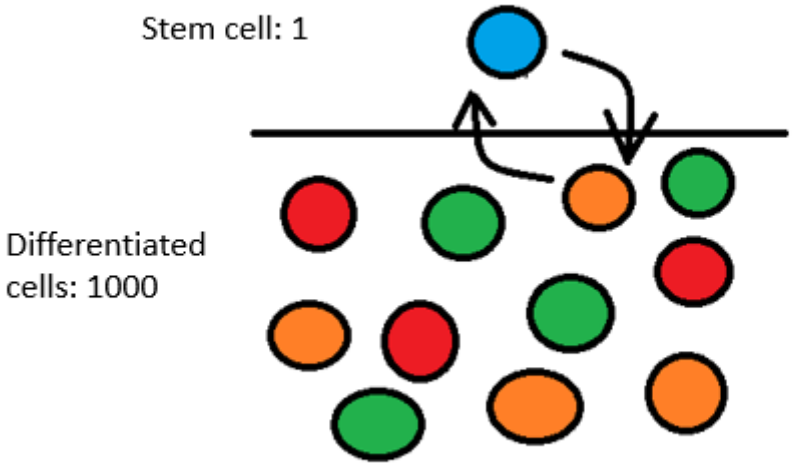

Figure 3: Stem cells and differentiated cells. The cancer stem cell (blue) switches place with differentiated cells (orange, green and red). This means any stem cell can become a differentiated cell and any differentiated cell can become a stem cell. Thus, a tumorigenic cell can become non-tumorigenic and a non-tumorigenic cell can become a tumorigenic cell.

Implications of the cancer stem cell model for future therapy

In the types of cancer that follow the cancer stem cell model, the differences between tumorigenic (possible stem cells) and nontumorigenic (differentiated cells) can have important implications for therapy. Evidence has been found that cancer initiating cells in certain types of cancer are resistant to therapy. For example in brain tumor initiating cells (S. Bao et al., 2006) and in breast cancer initiating cells (Diehn et al., 2009).While it is common to consider therapy resistance as a defining feature of cancer stem cells, it depends upon the type of cancer and the therapy how sensitive the tumorigenic and non-tumorigenic cells are to therapy. Some therapies exploit the property of tumorigenic cells to differentiate by inducing differentiation. For example, acute promyelcytic leukemia can be treated with arsenic trioxide and trans-retinoic acid. This induces rapid terminal differentiation, growth arrest and apoptosis of the tumor cells (de The & Chen, 2010). More types of cancer can be treated by inducing differentiation in cancer cells. Thus, some therapies already target tumorigenic cells. Cancer progression and cancer therapy resistance however, may be influenced by the characteristics of cancer stem cells in cancers that follow the model, but disease progression and therapy resistance can also be caused by genetic changes whether the cancer follows the cancer stem cell model or not.

However, the cancer stem cell model shows some flaws as mentioned before. If the cell-of-origin is not clear, the cancer cells to target will be difficult to find as the might not be very different from the other cancer cells. It will be necessary to find out if the type of cancer that is treated, follows the cancer stem cell model. If so, a targeted therapy has to be invented to treat this cancer. If not, it will be hard to target the cancer stem cells as they might not even exist in that type of cancer. In that case, other ways will have to be found to distinguish between tumorigenic and non-tumorigenic cells. It is also very important to find out if the cancer follows the cancer stem cell model because otherwise a therapy would target a small subpopulation which are not cancer stem cells. Patients would not benefit from this as the majority of the cancer cells will survive.

Recognizing cancer stem cells using markers

The characterization and identification of potential cancer stem cells could be very important for the development of cancer stem cell targeted therapies. Probably the best way to characterize and identify cancer stem cells is with the use of markers. In some types of cancer it has been proven that a small subpopulation of cells, which are suspected to be the cancer stem cells, have other characteristics than other (non-tumorigenic) cancer cells. For example, breast cancer stem cells were at first identified as a $CD24^{-/low}$ / $CD\ 44^{high}$ population (Al-Hajj, Wicha, Benito-Hernandez, Morrison, & Clarke, 2003). These cells show indeed an enhanced differentiation potential or self-renewal and/or are more resistant to therapy. Other studies also showed several other markers for several other types of cancer such as CD133 for brain tumor cells (Magee et al., 2012). Since then, several techniques have been proposed to characterize, identify and isolate cancer stem cells. This includes the sorting of cells by their surface phenotype through the expression of markers or through protein activity levels in a cancer cell. These can be measured using flow cytometry.

However, it is not sufficient to define a stem cell based only on surface markers. None of the markers used to isolate stem cells in different tissues and conditions are expressed solely by cancer stem cells. For example, CD133 which was found to be a marker for brain tumor cells, turns out to be also present in normal brain stem cells and on other non-stem cells in various tumors and tissues. It even turned out that both CD133 positive and CD133 negative cancer cells were showing tumorigenic activity (Magee et al., 2012). It is the same case with a lot of other markers such as CD44, THY1 and Sca1 (Clarke et al., 2006). The majority of cells that express these markers are not even stem cells. Also, markers which are used to identify stem cells from a particular tissue are most of the time not usable for identifying other stem cells in other tissues. Furthermore, if a marker is used to identify cancer stem cells from a certain type of cancer, it does not mean that it can be used in other types of cancer or in other patients with the same type of cancer (Clarke et al., 2006). Finding markers therefore is very hard to do. A found marker may or may not be useful for identifying other cancer stem cells in other tissues, types of cancer or other patients and that is a problem. To be able to prove the cancer stem cell model a marker has to be found which is only present in the small subpopulation of cancer stem cells. Only then the existence of cancer stem cells and the cancer stem cell model is really proven. In other words, it is important to be able to distinguish cancer stem cells from the other cells to be able to prove the existence of cancer stem cells.

As the cancer stem cell marker have been proven difficult to confirm in a broad number of solid cancer, the question arises if we might have overestimated the number of cancers that follow the cancer stem cell model. It also makes it very difficult to study the biology of these cells. On the other hand it is possible that a lot of cancers show a hierarchy structure but there could be a lot of diversity among patients in the markers that distinguish non-tumorigenic cells from tumorigenic cells. This is an important issue that has to be resolved. It may require other approaches than the traditional approach where it is dependent upon cell surface markers.

Cancer stem cell assays

Self-renewal and tumorigenic ability are the hallmarks of a cancer stem cell. Therefore, assays for cancer stem cell activity need to be judged for their potential to show both tumor propagation and self-renewal. Of course an assay can be performed both in vivo and in vitro. The best in vitro assay would be highly specific, quantitative, and sufficiently sensitive to measure candidate stem cells. Several assay have already been used to identify stem cells. These include sphere assays, label-retention assays and serial colony-forming unit assays. However each of these assays has some potential flaws (Clarke et al., 2006). For example, most cancer cells do not form mammospheres by themselves. Also the speed of sphere development in any of the systems makes it unlikely that they grew from single cells solely through clonal expansion. Serial colony-forming unit assays have been used to identify cells with increased proliferative potential but their activity has to be confirmed by a clonal in vivo assay (Clarke et al., 2006).

The best assay that fulfills the criteria is serial transplantation in animal models. This assay, although imperfect, is seen as the best assay for these two criteria. In transplantation assays, cells are xenografted into an orthopotic site of immunocompromised mice that are assayed at various time points for tumor formation (Clarke et al., 2006).To show and prove self-

renewal, cells must be isolated from the tumors and be xenografted into a second animal. The problem with transplantation assays is that there are potential effects of the grafting site. Cells are known to be highly dependent on signals from surrounding stromal cells and it is not sure what the effect might be on separating cancer stem cells from supporting cells during the course of the assay. The interpretation of this assay is also complicated because the cells in the tumor may form a lot of diverse cell types because of high genetic and epigenetic instability and not because they are stem cells per se. Even though serial transplantation assays are the best developed method to identify cancer stem cells, it has many flaws. A transplantation experiment can take 6 months or more for example. So although it is currently the best assay, more precise, sophisticated and simpler assays are likely to be developed as the field develops. This will significantly enhance drug development and a better understanding of cancer stem cells.

To identify the cells both in vivo and in vitro flow cytometry is used. It offers a specific, sensitive and robust method of cell isolation and purification. Unfortunately, some of the technical limitation makes it hard to purify stem cells. For example, doublets, cells sticking to other cells, can be sorted together and this needs to be eliminated. Therefore, microscopy is needed to know if single cells were indeed isolated. As it is very hard to make single-cell suspensions of solid tissues, techniques for sorting cells must be carefully developed. Because of all of these technical implications, an intense training of several months is needed to master flow cytometry.

Overview of arguments in favor and arguments against cancer stem cells

Arguments in favor of cancer stem cells:	Arguments against cancer stem cells:
A tumor is hierarchically organized where only a small population of cells is tumorigenic (Reya et al., 2001).	It is found in melanoma that the population that is tumorigenic is not small at all. 28% of cancer cells were able to form new tumors when transplanted in mice (Quintana et al., 2010).
Cancer stem cells can derive from both stem cells and normal, already differentiated, cells. This makes the possibility to form cancer stem cells larger (Magee et al., 2012).	When cancer stem cells can derive from all kind of cells, it could become difficult to identify and characterize the cancer stem cell population. This makes the cancer stem cell model more difficult to prove.
Marker are used to distinguish between cancer stem cells and normal cells. For example, a marker has been found in breast cancer to identify cancer stem cells (Al-Hajj et al., 2003).	Most markers are also present on non-tumorigenic cells or no markers are present at all. For example, no markers were found to enrich for tumorigenic cells in melanoma cells (Quintana et al., 2010).
A lot of assays exist to grow tumor cells and to find cancer stem cells. The best assay at the moment is serial transplantation in mice (Clarke et al., 2006).	Every assay that currently exists has flaws which makes it difficult to definitely prove cancer stem cells. For example, serial transplantation in mice takes 6 months and it is not sure whether tumor cells differentiate because they are cancer stem cells or because of high genetic and epigenetic instability.
Cancer stem cell assays improve over the years. For example in leukemia. For leukemia the assays are good enough to find cancer stem cells. This is a case where the cancer stem cell hypothesis is clearly proven (Taussig et al., 2005).	

Conclusion

As said before, cancer stem cells are a small tumorigenic subpopulation of a tumor. Whereas differentiated cells form the bulk of the tumor and are non-tumorigenic. The cancer stem cell model provides a model to explain cancer stem cells. Unfortunately, this model does not address the cell-of-origin. It is therefore difficult to say if this model is true and if it is applicable for all, some or none of the different types of cancer. It is very important for future implications to know if the cancer stem cell model is true and if cancer stem cells exist. Otherwise a small population of cancer cells will be targeted while this has no advantage for the patient. However, even though a cell-of-origin cannot be found, it is still important to know if heterogeneity exists in these different types of cancer. When there is no heterogeneity all cells have to be eradicated and can most likely be treated similarly. If there is heterogeneity within the population, the cancer stem cell population might be the most important part to be targeted. Markers are used to identify, characterize and isolate cancer stem cells. Unfortunately none of the found markers is found solely in cancer stem cells. They are also found in other cancer cells and other tissues which makes them hard to use. This makes the

cancer stem cell model difficult to prove. It means that a perfect marker has to be found which is only present at the cancer stem cells and not in any of the other tumor cells. To grow the cancer stem cells and to enhance for these markers, different assays are used. In vitro assay all show flaws which makes an in vivo experiment the best choice. Serial transplantation is considered to be the best assay available at the moment. However, this assay also is not perfect. The problem is that the transplanted cells are highly dependent from signals from surrounding stroma cells and it is not clear how this affects the cancer stem cells. So is there an answer to the question if cancer stem cells and the cancer stem cell model are real? For now, that remains a mystery. It is simply too early to say. However, it seems to be promising in at least a few types of cancer such as breast cancer. The biggest problem is that techniques are not developed enough to determine the possible cancer stem cell population. So when the field emerges and better ways are found to characterize and identify cancer stem cells, a better image can be formed about the cancer stem cells and the cancer stem cell model. For now, cancer stem cells remain a promising mystery and much work still has to be done.

Resources

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