

Chapter 2

Cellular Plasticity, Cancer Stem Cells, and Cells-of-Origin

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Abstract It is now recognized that tumor cells share similarities to stem cells in that they do not have a strong sense of functional identity. In the case of the skin, a mature cell that becomes stem-like no longer has the identity of a skin cell, meaning it changes shape and behaves in ways that make it not fit in with its normal skin cell neighbors. Accompanying these changes in behavior are changes in which genes are transcribed or repressed. This is why the transcriptome of cancer cells or cancer stem cells often share many similarities with the transcriptome of embryonic or adult stem cells.

Introduction

What is the point of understanding whether a tumor arises from one cell or a number of different cells? These are the cells that allow the tumor to regrow after treatment has shrunk it. The regrown tumor is often meaner than the original tumor. These are also the cells the leave the tumor and colonize other organs in the form of metastasis, which is often what kills a patient. Thus, if medical research wants to develop more specific therapies that have fewer side effects and a lower risk of relapse, knowledge of cells-of-origin or cancer stem cells is essential. Furthermore, as nanotech research and in situ live-imaging technologies seek to treat cancer, they cannot be guided to target specific cells and spare normal cells if we do not know the properties of cells-of-origin or cancer stem cells.

This chapter will discuss common concepts that are similar but not identical, which cause confusion within the field about how different studies relate to each other. For example, the cell-of-origin of a tumor may not be the same cell as the “cancer stem cell” (CSC) of the tumor. Furthermore, even if a tumor were derived from one transformed stem cell, there are processes within a tumor that would alter the identity of cells that arose from that initial stem cell, or that would produce hybrid cancer stem cells that may or may not resemble the initial stem cell.

Section 1: Defining the Terminology Necessary for Understanding This Field

To understand the concepts of cell-of-origin, cancer stem cell, dedifferentiation, and plasticity in cancer biology, it is important to clarify terms that are commonly used in the field and its various subfields.

Stem Cell

A stem cell is a cell that has two defining properties: (1) it can self-renew, meaning it can divide to produce two daughter cells, at least one of which remains a stem cell; and (2) it has the potential to mature (see the definition of “differentiation”) into multiple different types of cells. When a stem cell divides to produce a stem cell and a progenitor cell, this is called *asymmetric division*. If a stem cell divided to produce two stem cells, then this is called *symmetric division*.

Progenitor Cell

A progenitor cell is similar to a stem cell, except that it has less potential with regard to what cell types it can mature to become. Depending on the type of tissue, progenitors can self-renew like stem cells do, producing more cells that maintain an identity of being a progenitor. Progenitors might also undergo what is called transit amplification, meaning they divide rapidly to form more copies of themselves. These types of progenitors are referred to as transit amplifying cells. The purpose of transit amplification is to produce many progenitors from a few starting progenitors, such that they turn into many mature cells. This effect spares stem cells from having to divide many times. For example, one stem cell can divide once to produce a stem cell and a progenitor cell. The resulting progenitor cell can then divide many times to produce many progenitor cells, so that the original stem cell doesn’t need to divide unnecessarily. Depending on the organ, there can be early progenitors and

late progenitors. The further down the line a progenitor becomes as it matures, the less potential it has to become different types of cells, meaning the more committed it is to a certain “fate,” or identity.

Differentiation

Differentiation is the process in which a stem or progenitor cell matures into a cell with a specific function and identity. In cell biology and tissue biology, cellular function and identity go hand-in-hand. To speak generally, a stem cell that differentiates into one type of skin cell will take on the physical properties of skin and will not be able to do what a liver cell does for the body. Differentiation is how cells specialize in performing a certain duty, which requires them to turn on the genes and make the proteins necessary for that duty. The change in programming of what genes are turned on and which are turned off is an important reason why cells that differentiate into one type cannot differentiate into another type under normal circumstances. Differentiation is accompanied by a change in chromatin structure, epigenetic marks, transcriptomic profile, and cytoskeletal structure. A differentiated cell is a cell that has matured into a specific cell type that has a specific function.

Potency

This concept describes the potential that a stem or progenitor has for becoming different types of differentiated cells. The term *totipotent* means that a stem cell can become any differentiated cell type in the body. During mammalian embryonic development, a process called gastrulation occurs after which the three main germ layers are formed: endoderm, mesoderm, and ectoderm. Cells in these germ layer lineages are called *pluripotent*, meaning they can become multiple differentiated cell types that are derived from the same germ layer. For example, nerve cells, skin cells, and breast cells are all derived from the ectoderm. A *bipotent* progenitor cell is one that can only become one of two differentiated cell types within the same germ layer lineage. A *unipotent* progenitor is one that can divide to produce more of itself, but when its descendants differentiate, they can only become one cell type.

Lineage Commitment

This concept refers to the point of no return as a stem cell matures into an early progenitor and then to a late progenitor. The further along the progenitor goes along this continuum, the more it becomes committed to a certain fate, meaning the less potential it has to differentiate into different cell types.

Transdifferentiation

This describes the situation when a differentiated cell from one germ lineage (endoderm, mesoderm, or ectoderm) becomes a differentiated cell from another germ lineage. In mammals, this does not happen in a normal animal unless genetic engineering or tissue engineering is involved. Transdifferentiation is one of the main goals of adult stem cell research. For example, turning a person's skin cells (ectoderm origin), which are very abundant, into stem cells that can then be injected into that person's damaged heart (mesoderm origin) for the purposes of tissue regeneration.

Dedifferentiation

Dedifferentiation is the process in which a differentiated cell undergoes changes to lose its matured cell identity to become progenitor-like or stem-like. A dedifferentiated morphology is one of the hallmarks of cancerous cells. Dedifferentiation is accompanied by changes in cell-to-cell interactions, which allows the dedifferentiated cell to take on an odd shape or to divide "out of line" such that its daughter cells are no longer neatly packed like the other cells around them.

Plasticity

The concept of plasticity describes the potential of a differentiated cell to dedifferentiate back into a progenitor-like or stem-like state, and then to differentiate into a new differentiated state. The underlying concept is that a cell's identity, and thus its function, is not permanently fixed after a cell has differentiated. Within a tumor that contains many different regions of distinct morphology, or an early stage invasive tumor that is next to normal tissue, there are areas of transition showing groups of neighboring cells changing from one shape to another in ways that normal, differentiated cells do not do. Such drastic changes in cell morphology change the transcriptomic and proteomic identity of the cell. Thus, within the permissible environment of a tumor there is ample evidence of cellular plasticity.

Epithelial-to-Mesenchymal Transition (EMT)

Epithelial-to-mesenchymal transition (EMT) is a process in which an epithelial cell takes on the features of a mesenchymal cell, which allow it to break away from epithelial-type cell-to-cell interactions and to have enhanced migratory abilities. The function of epithelial cells is to form cube-like blocks that attached together to form a wall, which inherently limits their ability to move relative to mesenchymal

cells that form layers of interspersed, flattened cells. The process of EMT has been well-documented to give an epithelial cell stem cell-like properties, though this is not necessarily true in every case

Niche

A niche is defined as the location in which a stem cell or progenitor cell likes to reside and perform its function of making progenitors or more of itself. The niche is a very important concept in stem cell biology and cancer biology, because those who study tissue development, organ regeneration, wound healing, or metastasis all study at least one facet of how the niche regulates stem cell function. Why are stem cells in tissue located where they are? What activates a dormant stem cell to produce progenitors that heal a wound? Do stem cell-like circulating tumor cells prefer certain locations when they seed into and metastasize in distant organs? Are these locations natural stem cell niches or does the roaming stem-like cell induce its own niche? Is there such a thing as an inducible niche? If so, how should we understand and predict sites of metastasis? These questions are highly relevant for understanding the fundamentals of organ development, wound healing, and tumor development. An organism's niche within its habitat is also an important concept in ecology. If defined in detail, the concept of a niche draws together many of the topics that are studied in ecology (i.e. types of species interactions, physical or biological factors that affect population density, ways of using available resources, waste management, mating habits, etc.). The niche is one of the concepts that unifies ecology. The niche is also a concept that unifies the topic of this chapter: plasticity, cancer stem cells, and cells-of-origin. A niche isn't just a basket of extracellular matrix proteins in which a stem cell sits. In a niche, the stem cell is surrounded by neighboring cells that communicate with it. These cells often play very important roles in regulating the activity of the stem cell. A stem cell in a niche can be affected by physical contact with its neighbors, paracrine signals released by its neighbors, signals from cellular projections that reach over from a distance, among other mechanisms of cell-to-cell communication. The reality, as with other aspects of biology, is likely multiple simultaneous signals.

Microenvironment

The microenvironment refers to physical attributes surrounding a cell, which includes the extracellular matrix molecules, temperature, pH, salinity, hormonal milieu, electro-magnetic signals, connective tissue cells (fibroblasts, blood vessels, lymph vessels), and immune cells that are present. The term microenvironment is often used to describe a general condition of, within, or surrounding a cell/tumor; often a condition that promotes a specific behavior of the tumor.

Clonality

This concept is important in discussions of cancer stem cells and cells-of-origin because it underlies part of the reason why certain therapies shrink a tumor, only to have the tumor return. A clone in this context is defined as a single cell that divides to give rise to a large population of identical cells. Polyclonal describes a tumor or population of cells that is derived from multiple distinct clones. Monoclonal describes a population of cells that is derived from one cell.

Cancer Stem Cell

A cancer stem cell (CSC) is a cell that divides to replenish a population of cancer cells. CSCs can exist in cell lines that are grown in two-dimensional and three-dimensional culture systems or in tumors that are grown in living organisms. CSCs are the cells that survive a therapeutic treatment that destroys the vast majority of the tumor. CSCs divide to produce cells that make up the returned tumor. CSCs have special properties that allow them to be distinguished from non-CSCs. CSCs are better able to repair damage that occurs inside of them, such as DNA and protein damage. They are also better able to quench reactive oxygen species inside of them, reducing the amount of damage that they incur when under stressful conditions. CSCs are often the cells that leave the primary tumor, survive in the blood stream, and metastasize distant organs. The terms cancer initiating cell (CIC) or tumor initiating cell (TIC) are often used to describe cells that are essentially CSCs, but are named differently because of how they were identified or tested as having stem cell-like properties. Section 2 of this chapter describes common confusions between CSCs and cells-of-origin.

Cell-of-Origin

A cell-of-origin is a normal stem or progenitor cell that becomes abnormal and gives rise to a cancer, liquid or solid. Due to its origin from a normal cell, the cell-of-origin is not necessarily the same thing as a cancer stem cell, though it may be. Cancer stem cells were derived from studying tumors, while cells-of-origin are identified by lineage tracing experiments that can track normal cells that become part of tumors. Section 2 of this chapter describes common confusions between CSCs and cells-of-origin.

Section 2: Confusion Among Stem Cell, Cancer Stem Cell, and Cell-of-Origin Concepts

The purpose of understanding cancers stem cells and cells-of-origin is to allow for the development of more specific therapies and therapies that result in less relapse. However, discussions of these two topics often results in confusion, since their

definitions can overlap. Clarity on this matter is important because cancer prevention strives to keep normal cells from becoming cancerous, while cancer treatment seeks to eliminate cells that are already cancerous. The point of cancer research at the molecular and cellular level is so that these details allow us to produce better therapies that have fewer side effects. Clear definitions of function and identity are necessary for nanotechnology and targeted drug delivery methodologies to specifically target CSCs or cells-of-origin, while sparing normal cells.

The microenvironment of the tumor is drastically different than a normal tissue. Thus, even if a tumor arose from one cancerous cell among normal neighbors, by the time that one cell has divided enough times to form a tumor within that tissue, the microenvironment of that tumor may have turned non-CSCs into CSCs. Therefore, a tumor that started from a transformed normal stem cell, the cell-of-origin, may harbor CSCs that did not come from that stem cell. A tumor may harbor any combination of cells-of-origin and CSCs. This is because as a tumor grows, it enlarges and engulfs neighboring areas that also have normal stem cells. Those engulfed stem cells may then become additional cells-of-origin. Furthermore, the engulfed normal differentiated cells may become additional CSCs. Thus, a tumor may contain multiple cells-of-origin and multiple CSCs, each of which joined the tumor at different times. Given the complexity of the combinatorial possibilities of cells-of-origin and CSCs within a tumor, individual research papers need to assume simplifications in their conclusions about cells-of-origin and CSCs. However, for both those who are advancing this area of knowledge and those who are new to it, being aware of this complexity will help reveal new mechanistic insights, organize existing literature and future knowledge, and guide hypotheses.

Section 3: Evidence for the Theory of Cell-of-Origin as the Root of Tumors

Much has been written about the evidence for the cell-of-origin hypothesis. The reader is directed to the two excellent reviews that are briefly discussed here. The first is by White and Lowry (White and Lowry 2015), which discusses studies that provide evidence that specific populations within the hierarchy of stem cells and progenitor cells can be mutated to give rise to specific subtypes of cancers. The studies that were discussed yielded slightly different stem or progenitor cells as the cells-of-origin within hair follicles and the cells surrounding them. However, the clear uniformity from these studies is that causing mutations in a specific cell type within the stem cell hierarchy produces a specific type of tumor. While this supports the cell-of-origin theory, it is important to keep in mind that these studies are not yet able to trace the origin of the heterogeneous tumor—that is, one that has multiple distinct regions of morphology or behavior—to one or a few cells. The second review is by Jane Visvader (Visvader 2011), which outlines several conceptual models of how stem cells are mutated to become cells-of-origin. This review also discusses studies that support the cell-of-origin hypothesis. The best support comes from studies of hematopoietic cancers in which over expression of oncogenes in various

compartments of the stem cell hierarchy resulted in cancer. The upper/earlier compartments, that is the stem cells, are more susceptible to oncogene-induced transformation that result in cancers, while the progenitor compartments are not. However, a higher dosage is able to produce cancer from the progenitor compartments, which highlights that given a permissive environment, such as a tumor, the right mutations can arise in cells that do not normally serve as cells-of-origin such that they become cells-of-origin. Further evidence comes from mouse models of intestinal cancer, prostate cancer, basal cell carcinomas, and pancreatic ductal cell carcinomas.

Section 4: Evidence for the Theory of Cellular Plasticity in Tumor Development

The cellular plasticity exhibited by partially or fully differentiated cells reverting back to stem cell is an alternative hypothesis about how tumors develop, especially those that harbor heterogeneous cell types. The liver is an organ that can regenerate itself after being wounded. Progenitor liver cells are believed to exhibit reversion back into stem cells even while in a non-cancerous—albeit wounded—organ state, in order to regenerate the region that is missing due to hepatectomy (Choi et al. 2014; Dorrell et al. 2011; Huch et al. 2013; Santoni-Rugiu et al. 2005). This type of plasticity is also observed in cancer. Klevebring and colleagues (2014) sequenced the exomes of CSCs and paired non-CSCs from breast cancer patients. To their surprise, the two populations shared the majority of the same somatic mutations, which argues against the idea that CSCs have a distinct mutational profile because they don't divide—and accrue replication errors—as much as the bulk of the tumor cells that arise from them. These data suggest that CSCs may be switching between a CSC and non-CSC state, since they share such similar somatic mutation profiles. Chaffer and colleagues (2011) made a discovery that also echoes the cellular plasticity theory. They found that differentiated human breast epithelial cells could spontaneously revert to a stem-like state. Furthermore, oncogenic transformation of these differentiated cells increased the rate of reversion to the stem-like state, producing CSC-like cells in vitro and in vivo. The plasticity of cancer cells is made extra evident in the study by Sharma and colleagues (2002), which showed that epithelial prostate cancer cells could dedifferentiate and then become vasculogenic cells that comprised blood vessels inside of tumors, something that epithelial cells are not supposed to do.

Section 5: Confounding Factors in the Plasticity Versus Cell-of-Origin Debate

It is difficult to clearly prove that a tumor arose from and is sustained by purely one cell-of-origin without the addition of CSCs that were derived from cells other than that cell-of-origin. If Sections 2 and 4 of this chapter did not make this point obvious, this section will further solidify it.

Cell Fusion

One major confounding factor in proving that a tumor is derived from one cell-of-origin is that spontaneous cell fusion can occur. Terada and colleagues found that mesenchymal stem cells from the bone marrow of mice could spontaneously fuse with mouse embryonic stem cells (Terada et al. 2002), which has deep implications for what the mesenchymal stem cells can do when they encounter CSCs or non-CSCs within the tumor microenvironment. Bone marrow-derived mesenchymal stem cells are common components of tumors. A study by Ying and colleagues further implicates the importance of cell fusion events that occur when stem-like cells interact. The authors isolated neural stem cells that were engineered to have permanent genetic markers (green-fluorescent protein and puromycin resistance), and then cultured them with mouse embryonic stem cells that were engineered to also have permanent genetic markers (hygromycin resistance). They discovered that the neural stem cells could spontaneously fuse with the embryonic stem cells to produce hybrid cells that contained genetic markers from both cell types (Ying et al. 2002). The publication of the Terada et al. and Ying et al. papers required a reinterpretation of a number of transdifferentiation studies in which cell fusion may actually have been the mechanism, instead of transdifferentiation, by which the cells' phenotypic identities were altered (reviewed in (Wurmser and Gage 2002)). In 2013, Lazova and colleagues reported the first case of cell fusion in human cancer. By measuring regions of DNA that were unique to the recipient or donor from whom bone marrow was taken, the authors were able to show that the melanoma that metastasized to the brain of the recipient was a hybrid of both the recipient and the donor (Lazova et al. 2013).

Induced Mesenchymal States

Epithelial-to-mesenchymal transition (EMT) is a process in which an epithelial cell takes on the features of a mesenchymal cell, which allow it to break away from epithelial-type cell-to-cell interactions and to have enhanced migratory abilities. The function of epithelial cells is to form cube-like blocks that attach together to form a wall, which inherently limits their ability to move relative to mesenchymal cells that form layers of interspersed, flattened cells. The process of EMT has been well-documented to give epithelial cells stem cell-like properties, though this is not necessarily true in every case (reviewed in (Brabletz 2012)). Much has been written about growth factors and over-expressed genes that induce an EMT and CSC phenotype.

This section will discuss a mechanism of cell-to-cell communication that allows for the transfer of nucleic acid polymers and its implication on bulk tumor gene expression profiling data. Exosomes are microscopic membrane vesicles that bud off from a donor cell and that can fuse with another cell, dumping their contents into the recipient cell. The discovery that exosomes can deliver nucleic acid polymers, such as microRNA (miRNA), which can alter the transcript level of their target genes in the recipient cell (Melo et al. 2014; Singh et al. 2014; Yang et al. 2011) has

important implications for understanding mechanisms that induce cellular plasticity. These mechanisms of nucleic acid transfer make sense of why tissues that seem morphologically normal to a pathologist can have a stem cell-like or progenitor-like transcriptomic signature that is detected by mRNA extraction from bulk tissue. Spatially guided, laser-microdissection transcriptomic techniques that allow the investigator to identify the transcriptome of distinct regions or cells within a sample may clarify epicenters of exosome-mediated nucleic acid transfer (reviewed in (Crosetto et al. 2015)).

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