In this tutorial, I'm going to cover some of the basics of what stem cells are and how they function throughout development. One of the best adjectives I can use to describe stem cells is the word 'potential' because stem cells hold the functional potential to give rise to an array of cell types, even the entire embryo and everything needed to generate a multi-cellular organism. But they also hold amazing potential for medicine. Stem cells have permeated our society in so many ways and unfortunately, many misconceptions exist in the public domain. Let's start with the first question. What is a stem cell?

In order to be classified a stem cell, you need to satisfy three main criteria. The first one is, stem cells are generally regarded as undifferentiated or unspecified, and as such retain the ability to divide. The second property is that upon division, they have the ability to self- renew, meaning make themselves again. And the last criteria, also upon division, the daughter cells can go on to mature, and differentiate into a variety of cell types, depending on the stem cell and tissue type of origin.

While all stem cells satisfy these three main criteria, they can differ in several different ways. And really, fundamentally, those ways are based upon the idea of potency. And what I mean by potency is, the number or amount of different cell types that that particular stem cell type can generate. So, the first category is totipotency, in which would pertain to primarily the single-celled zygote. So, just after fertilization, that single-celled zygote retains the potential to of course, give rise to an entire organism. But there are two principal aspects of an organism. The first one is the actual embryo itself, but also the supportive material called the extraembryonic tissue. So, a zygote, and really, the cells after the first few divisions of that zygote, are all capable to generate extraembryonic tissues and the embryo proper.

The next category, and, as I move through these three categories, we're becoming more restricted in the potential of what those stem cells can do, is pluripotency. The only naturally occurring stem cell that is considered pluripotent are embryonic stem cells. Embryonic stem cells are only found at a very early stage in development called the blastocyst and within the blastocyst, there is an inner cell mass, and it is only in that

inner cell mass where stem cells, as being pluripotent, are capable of generating the entire embryo. So, all the cell types that make up all three germ layers, ectoderm, mesoderm, and endoderm, can be derived from embryonic stem cells. However, embryonic stem cells, at least, as we know it now, cannot generate extraembryonic tissues. Some evidence that embryonic stem cells can actually generate the entire embryo may actually be sitting right next to you. And that is, twins. So, here is an image of an inner cell mass of a blastocyst, and naturally, this inner cell mass can split into two sometimes. And when it does that, it has the ability to ultimately develop and generate monozygotic twins.

Another example, inner cell mass cells, those embryonic stem cells, and transplant them into a mouse. Into an adult mouse. Into the body wall or sub-cutaneous tissue of that mouse, and those embryonic stem cells will divide, and when they do so will generate a tumor. These particular tumors are called teratocarcinomas, or teratomas. And when you take that tumor out and analyze it, you can actually see cell types and tissues derived, and representative of all three germ layers - ectoderm, mesoderm, and endoderm. Again supporting the fact that these embryonic stem cells are pluripotent, and can generate everything that the embryo needs.

The third category of potency is multipotent, and there are a variety of different stem cells that fit within this category. But generally, they are all called adult stem cells or somatic stem cells. Adult stem cells show what you would consider greater lineage restriction to the cell types that they can generate. So, there are a variety of different multipotent stem cells, and both in the embryo, fetus, and adult, these multipotent stem cells really reside within specific organs or tissues. And the lineages that they can generate, I mean the progeny and cell types that they can create, are really restricted to those organ types.

Here you see just two examples of two different pools of developing multipotent stem cells, one in the brain, here's an image of an adult neuro stem cell nestled within the hippocampus. And then, over here is my illustration demonstrating where the bone

marrow is within a bone, and that would be the amanopoetic stem cell where it exists to generate a variety of cells within the blood and immune system. So the adult stem cell, whether they're coming from brain, blood, bone, lung, gut, skin, teeth, they will oftentimes remain quiescent until the environment shows that there is stress or injury and there is a demand for increased cells. And then they will initiate a state of activation, and undergo proliferation and begin to divide, whatever insult or stress that is currently existing. In fact, all organs and tissues that have been examined have found stem cells in them. They're very limited in number, and actually reside in a very specific location called the stem cell niche. And, every organ and tissue has their own characteristics of that stem cell niche. It is the stem cell niche, the cells within it, that support that stem cell will ultimately influence whether that stem cell remains quiescent or undergo division.

Lastly, I also mention in contrast to embryonic stem cells, adult stem cells are considered to have a shorter life span, at least in culture, whereas embryonic stem cells will seemingly divide and proliferate indefinitely. So, what I'd like to do now is talk a little bit more about the main behavior of stem cells, that of division, and potentially how it's regulated, primarily in the adult stem cell niche. So, if all a stem cell has to work with is division, then what are its options?

Well, if a stem cell goes to divide, in that division it will generate two daughter cells. Right there is option number one. What do those two daughter cells go on to do? As I mentioned stem cells have the ability to self-renew. So they can divide and simply make two more stem cells. However, one of those daughter cells may begin to become specified and start to mature along a differentiative path, whereas the other one remains as a self-renewing division. So what we would call that is an asymmetric self-renewing division. Where one daughter cell generates another stem cell and the other daughter goes on to differentiate in this example into a neuron. But what if both of the daughter cells actually began to become specified and differentiate? That would be a symmetrical differentiating division in which both of the resulting cell types get generated. In this case two neurons. However, as I mentioned, you can have a symmetrical self-renewing

division and what that will do is help to increase the stem cell pool. Many times throughout embryonic development that's going to be really important to generate larger sources of those multipotent stem cells that can help to build the tissues and organs during organogenesis. However, I want to mention that what can happen as these stem cells are undergoing many, many rounds of proliferation is that mutations can occur and if one mutation were to occur in a particular stem cell in which would perhaps foster greater levels of proliferation or rates of division or inability to remain quiescent, well then you run the risk of tumor formation and ultimately cancer.

In a majority of the congenital brain tumors that are formed have been linked back to those early neural stem cells. So a next logical question is what regulates these divisions? One of the major modes of regulating stem cell division lies in cell to cell communication. Signals from the environment will be interpreted and received by that stem cell and those will ultimately provide the instructions to undergo division and start to divide. These would be considered mitogenic signals, signals that ultimately promote proliferation.

The other major influence of how stem cells choose to divide whether symmetrically or asymmetrically, lies in the actual mechanics of division, that mitotic spindle. And in particular, the plane in which that mitotic spindle is oriented. In some planes it will foster a symmetrical division, while in others, it will foster an asymmetric division. This is often times paired with a very important factor intracellularly and that factor will become asymmetrically localized to one of the daughters, as opposed to the other. That will ultimately promote an asymmetric differentiating division, where there is a self-renewal, and a maturing daughter cell. It doesn't necessarily happen where a stem cell will ultimately divide, and that progeny will magically turn into that differentiated cell type, but it's oftentimes a longer transition of maturation that can occur over successive generations of proliferation. I've sort of implied that stem cells generate differentiated cell types, in a way that perhaps suggests they go directly into that cell. Well, that's not really what happens. In most cases, this is a transition period of maturation and in that process, they generate progenitor cells. So, here is a stem cell right here that I've

represented. That stem cell will divide and then, following that division in this example, making an asymmetric differentiating division such that one is self-renewing. And then, this purple cell here is one in which is going to go on to mature and become what we would call a progenitor cell. Now, this progenitor cell retains the ability to divide itself, which I'll illustrate with this symbol. When it divides, it will generate a daughter cell and another daughter cell. These two daughters can in fact be derived from a self-renewing division, a symmetrical self-renewing division so that they too can begin to divide. This is an important mechanism that will help to increase a targeted population of cells, typically during embryonic development.

However, there are other organs that need to regenerate, such as in the blood or in the skin. And in those cases, these progenitor cells will continue to operate in this way to generate the differentiated cell type they need to for that tissue. But in this example, here we have a population of progenitor cells proliferating. They will though, begin to mature and when they mature, start to transition into what we would call precursor cells until they develop into the differentiated cell type. In this example, this one developing into a neuron, however this progeny, developing into an oligodendrocyte, which is a glial cell type within the nervous system. So this original parental stem cell divides through an asymmetric differentiating division, generating a progenitor cell, here, purple, which itself could divide making more self-renewing progenitor cells until those reach a point where they become precursor cells and will begin the true differentiative path toward the lineage where they find themselves, often times due to the position that they're in. So the position where this blue progenitor cell finds itself may be different than this red progenitor cell. And consequently, the environmental cues in that location perhaps different. Over here, we have signals that will ultimately direct this cell to develop into oligodendrocyte and over here those signals are absent, so this one develops into a neuron. And in that way, you go from adult stem cell to differentiated cell type, over the course of successive generations of proliferation and maturation.

OK, what I'd like to do at this stage is take a step back and do a quick little review here about potency, or the potential that these different stem cells have to develop into

different things. The first one in the series of course, is the totipotent cell, or in this case, really just the zygote. That single cell zygote has and retains the ability to generate everything necessary to produce an organism - the embryo proper, as well as all extraembryonic lineages. Then we move down the potential scale to pluripotent. The only cell type there is the embryonic stem cell. Resides in the inner mass of the blastocyst, and it can generate all the cell types of the three different germ layers that make up the embryo. During development, it will create a series of multipotent stem cells associated with every tissue and organ. These multipotent stem cells can still divide, but have more lineage restricted ability to the cell types that can differentiate.

From multipotent stem cells, those will oftentimes operate through progenitor cells, those progenitor cells will also continue to divide and proliferate for a reduced period of time, sort of in a transient way, leading to the creation of multiple cell types that will then go on and mature into differentiated cells. So from your progenitor cell, you can give rise to precursor cells. That term, precursor cell, is often used but those cells typically are not dividing but are sort of an immature state leading to that differentiated cell type as illustrated here.

The last question I actually want to try to address is how is the adult stem cell sort of regulated to remain quiescent or begin to divide. And we've already mentioned that these adult stem cells reside in specific stem cell niches according to the organ or tissue that they are part of. And it is that stem cell niche that is ultimately going to influence those different states of stem cell division and behavior. So illustrated here is my representation of a stem cell, adult stem cell, and in these adult stem cell niches, they will oftentimes be directly adjacent to, or in fact, directly contacting, another cell type. Often times through cell to cell adhesion molecules that help to facilitate that connection. In addition, another really important factor are the extracellular matrix components around this stem cell and throughout the stem cell niche. These extracellular matrix factors can have proteins housed within them and those proteins can function as signaling factors that will influence the division states of that stem cell. Another really critical aspect of how that extracellular matrix can change is in its

biomechanics and physical properties of how flexible it is. And that biomechanical stress around that adult stem cell can also influence whether it remains quiescent or goes on to divide. They'll be other cells in the environment that, within this microenvironment of the neural stem cell niche, these cells will serve to secrete peracon factors, or growth factors, and in doing so, will establish different kinds of signals that can promote proliferation such as say this red signal, or, perhaps, repress proliferation, and keep things quiescent.

There may be additional signals from these same cells that help to facilitate differentiation. All of these combined can regulate the division states and what is happening within this niche. Moreover, you have, potentially, neural signals, neurotransmitters being released into the environment of this niche, that can also stimulate things, as well as the all-important blood vessel. And signals derived from that blood vessel will surely play a major role in influencing the states of this neural stem cell niche. But most important is what is happening to the daughter cell as it moves through this array of signals. That stem cell divides, generates its daughter, and that daughter will potentially migrate out of the niche or actually successively get pushed out of the niche through further proliferation of the stem cell. And in doing so, it begins to move out of the range of a lot of these other signals, ones that are telling it to proliferate, and perhaps move into a range of signals that are telling it to, in fact, differentiate. And in that way, the niche is a functioning environment in which all of the cells, including the stem cell itself, play vital and important roles.

Much research is being focused on trying to understand what the different signals are, who the different players are that are influencing these very important stem cells, and the differentiation of their daughters. Well, that concludes this tutorial, but I want to mention that we barely scratched the surface of stem cell biology. There are a variety of different stem cell types that I haven't discussed, whether it's neural crest cells and radio glial cells in the embryo, fetal stem cells, or the multitude of other adult stem cell types for different organs. One of the most important stem cell types that I have not discussed are called induced pluripotent stem cells. IPSCs possess the same functional

capabilities as embryonic stem cells, but are actually derived from a re-programmed, differentiated cell type. These are truly amazing cells, and their derivation has won the Nobel prize. What IPSCs offer is potentially patient-specific therapies, and the ability to study human diseases like we've never been able to do before. Scientists are manipulating and using stem cells to treat, and ultimately cure, human diseases, regenerate whole organs. I truly believe that we are living through a remarkable time of scientific discovery, one in which stem cell biology is going to truly revolutionize medicine as we know it.