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Editorial

Mesenchymal Stem Cells - What is in the Name?

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Since more than 40 years ago Friedenstein first described cells derived from bone marrow stroma that were plastic adherent and had the ability to differentiate into other lineages, our knowledge about stem cells has constantly been growing over the last decades. Nearly 10 years ago a definition was given by Dominici et al describing Mesenchymal Stem Cells as being plastic adherent, expressing CD73, CD90, CD105 and having the ability to differentiate into osteoblasts, adipocytes and chondrocytes. Since this time the expression "Mesenchymal Stem Cells" has been used to describe what we believe are multipotent cells. However, many questions have arisen over the last couple of years. In the beginning there was the assumption that only embryonic stem cells would be truly pluripotent, meaning they could differentiate into ecto-, endo and mesodermal lineages. Given all the concerns with embryonic cells both from an ethical, political but also from a practical point of view, research with embryonic cells was limited and partly created a lot of concerns, not only in the medical and the clerical community, but also among researches.

Therefore, the idea to induce pluripotency by overexpressing embryonic genes such as c-Myc, KLF4, NANOG, Oct4 or SOX2 was brought forward and was enthusiastically welcomed by the research community.

Researches in California were able to convince the politicians that this could be the new wave of a commercial success for the state of California and therefore billions in research money have been provided in order to support induced-Pluri-Potency (iPS) based research. Even the nobel prize was awarded for this assumed "break through" finding in the believe the need for embryonic cells could be overcome by using adult cells and by upregulating their embryonic genes.

A whole business has been established around and many researches depend on grant money provided for that assumed fantastic idea.

However the reality is: iPS cells are very good to study cellular development, they might be good for evaluation of new medication in a dish in order to test new developmental molecular pathways for drug therapies, but they definitely will not make it into the practice of Clinical Medicine. The reasons are pretty simple and should be obvious.

Nature has secured the survival of mankind by not allowing our stem cells to differentiate into an organ or a lineage which is not already present in the body. In an adult organism only cancer cells are able to form new cells independent from the local micro environment.

The Scanning Electronic Microscope picture was taken 15 minutes after the recovered cellular composition was plated on an artificial extracellular matrix.

We recognize the variation of the different cellular surfaces, size and structure. Lymphocytes are

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naked on their surface, while progenitor cells are larger than the early and small stemcells. Also, the cells clearly show the exosomeson their rougher surface, and the nanotubular structure between two progenitor cells. Both structures serve the exchange of genetic information between cells.

A good example is tumor metastasis in the liver by a metastatic pancreas cancer cell. Pancreatic metastatic cells don't care about the missing micro environment of the pancreas and continue their differentiation irrespective of the wrong local hepatic clues that will normally prevent the differentiation of a pancreatic cell in the liver.

This missing correct tissue specific microenvironment would normally prevent differentiation of pancreatic cells of the liver. This is the protection that our evolution has given us: embryonic stem cells are able to form an organ which is not present, yet.

Adult stem cells are pluripotent and can form every organ but under one condition: The maturing progenitor cell need clues, hints and guidance from the existing tissue. (**Fig. 1**) It is not enough that a cell might be already a progenitor cell; to become a fully functional adult cell it needs guidance, support and reconfirmation. We have shown this in a simple experiment: we extracted the nuclear proteins from rat cardiomyocytes and transfected human stem cells that were derived from adipose tissue with this nuclear extract. After 3 weeks the cells start to express the line of cardiomyogenic differentiation including not only an expression of cardiomyogenic genes in the development of a cardiac pathway, but also form proteins like Titin and structural proteins such as Alpha-Actinin and Myosin light chain.

However, cells that lack repetitive reconfirmation that they are on the right pathway in their differentiation, stop maturing and don't fully mature to adults cells. This is a valuable and important protection and also part of the safety of stem cells therapy.

The longest known clinical results with the use of stem cells are the ones with cells derived from bone marrow. Most people are not aware that more than 99% of those cells are progenitor cells on their way to become hematopoietic mature cells. An injection into a different organ such a knee would normally create a massive inflammation because if the leucocyte, lymphocytes, macrophages and monocytes continue their "Education" and become mature cells, it would end up in an inflammatory joint. However, the lack of reconfirmation in this "Non Bone Marrow" microenvironment prevents those cells from continued differentiation into a lineage which is not supported by the local micro environment. Only those cells which are early in their development and are still able to adapt to the novel micro environment, understand the clues that the

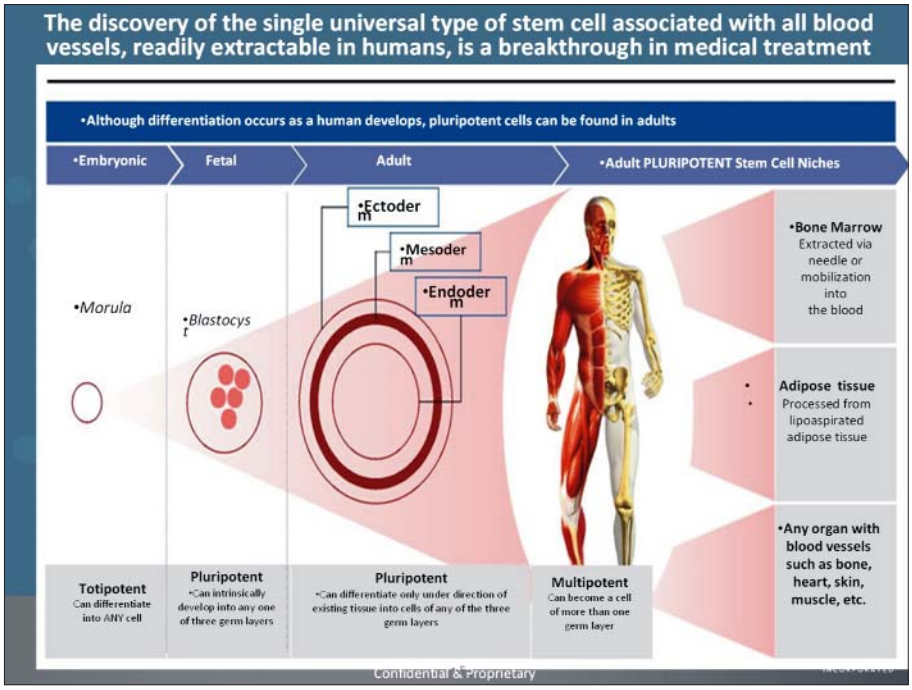

Figure 1.

micro environment provides by exchange of cytokines, and especially by exchange of microsomes that encode and encapsulate micro RNA and transcription factors, only these cells are able to follow that guidance and mature through different progenitor stages to a lineage, which is determined by the new environment.

However, the nature of cells which are able to differentiate and have pluripotent properties currently is not fully understood. Every tissue contains those cells - which form a reserve army - that is based in our blood vessels.

Therefore, the name Mesenchymal Stem Cellis correctas far as their orgin from mesenchymal tissue is concerned. These cells are silenced, are very small about 5 micrometer large cells (**Fig. 2**) and are sitting in the vessel wall and perivascular. In addition, each tissue has its own set of these ubiquitous immature pluripotent cells. In addition, each tissue has its specific progenitor cells, that have already left the very early undifferentiated state of small stem cells, and have taken the differentiation path of lineage commitment, but are still not fully matured. In so far, the term Mesenchymal Stem Cells is justified with regard to their orgin.

But do we really want mesenchymal cells as the aim and as a result of stem cell therapy? Mesenchymal cells mean in principal fibroblasts and connective tissue. Indeed, for a long time the difference between fibroblast and stem cells was a little bit obscured. In cell cultures stem cells are described as having a fibroblast like plastic appearance and attachment to the plastic culture surface. Several years ago the question to our research group arose what is the difference between stem cells and fibroblast, especially as they both look the same? Is there a way to discriminate stem cells from fibroblast just by their appearance? The answer is no. The next question is: are cells surface markers such as CD44, CD73,CD90, CD105 able to discriminate stem cells from fibroblast? The answer again is no. So the question is: How can stem cells and fibroblast be discriminated from each other?

We described two main differences. First of all: fibroblast don't produce the cytokines that are responsible for the antiapoptotic life saving beneficial effect of stem and progenitor cells. Stem cells produce primarily two important cytokines namely: IGF1 and VEGF that are first responsible both for the strong antiapoptotic effect of stem cells and secondly are responsible for the angiogenesis induced by stem cells.

A fibroblast is not able to produce these two cytokines. In addition, fibroblast are fully differentiated cells that have a purpose in life. There is no need, but also no ability to differentiate into

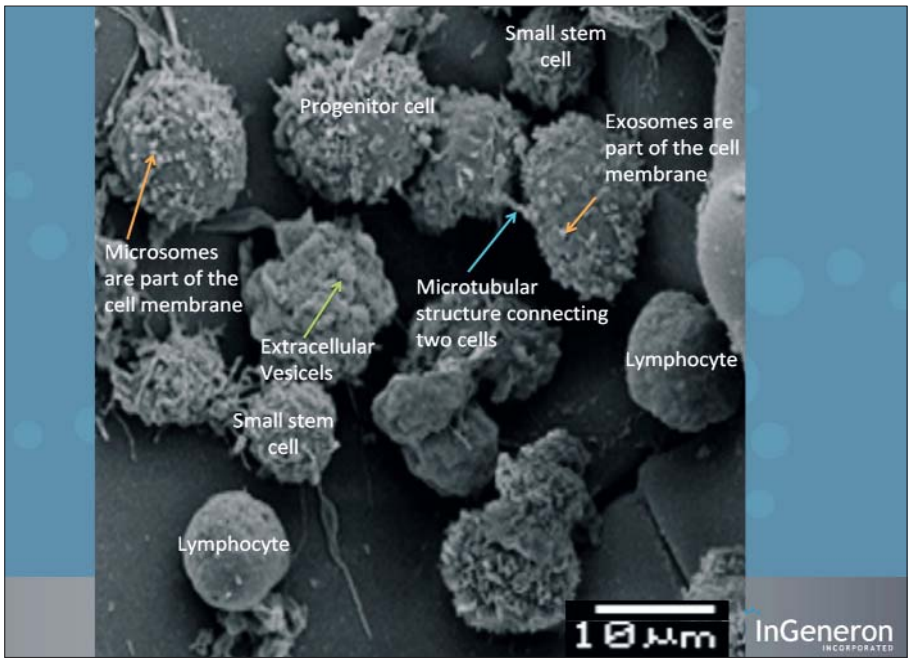

Figure 2. Stem and progenitor cells (together called Regenerative Cells) derived from processing of adipose tissue with an enzyme reagent containing Collagenase 1, Collagenase 2 and a recombinant Neutral Protease

other cell lines even if these cells are exposed to a lineage inducing cocktail.

Stem cells in contrast are able to differentiate into cartilage, bone, adipose tissue, skin, nerves, and liver indicating that there are able to differentiate into all three germ layers. And this without induction of so called pluripotency or iPS. These early stem cells are found in the wall of all blood vessels in every tissue, and independent from their origin, they are equally able to differentiate into all three lineages. The induction of iPS is a superfluous and unnecessary measure which make those iPS cells less safe. Several laboratory results and first clinical studies indicate malignancy among those cells.

As one of the leading iPS researchers in California states:

"I believe that iPS cells and cancer cells are, while not the same, close enough to be called siblings. As such, the clinical use of iPS cells should wait for a lot more study. Even if scientists do not use iPS cells them selves for transplants, but instead use differentiated derivatives of iPS cells, the risk of patients getting malignant cancers cannot be ignored".

If you expose for example early stem cells to the iPS inducing cocktail and overexpress embryonic genes, their induction to liver cells ends upin formation of malignant juvenile hepatoblastomas.

Unfortunately, most researchers are biased to keep their grants and don't report those negative results. The reporting about the true nature of iPS cells is obscured and driven by considerations that are even at times of limited research support to be called at best "unethical". The election of some of the inventors of iPS cells for the Nobel Prize doesn't change this.

Now coming back to the question: Do we really want mesenchymal cells as our aim in therapy? The simple answer is no. We don't want a repair with fibroblast and formation of a scar. Nature is doing that by itself if there is a lack of sufficient truly pluripotent stem cells that would be able to repair a damaged organ. What we truly want are parenchymal cells. In the language of the pathologist "Parenchym" are the cells that are building a functional organ. With stem cells therapy we don't want the replacement of missing parenchym by mesenchymas this is only connective tissue. What stem cell therapy really aims to is to replace a failing organ: we want to build heart, kidney, lung, liver, cartilage, bone, nerves, and replace the missing or damaged parenchym. Therefore, as far as the aim of stem cell therapy is concerned the term Parenchymal Stem Cells would fit much better our expectations and reflect the real clinical need.

Further research in this direction will certainly guide us the right way, for the final benefit of all patients in need of Regenerative Medicine with Stem Cells.

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