PRESS RELEASE

"Reprogramming" the identity card of cells: the molecular imprint has been identified

An all-Italian study marks a step towards the ultimate goal in regenerative medicine: therapeutic use of stem cells obtained through cellular reprogramming. The mechanism for reprogramming cellular identity involves proteins of the Polycomb family, already known for their role in embryonic development and tumor formation; they provide the obligatory "imprint" for inducing adult cells to return to the embryonic stage. This is demonstrated by a study conducted by scientists Stefano Casola and Giuseppe Testa in the laboratories of IFOM and IEO in Milan. The results, published today in *PLoS Genetics*, confer a significant advancement to our knowledge of cellular reprogramming, with the aim to fulfill the expectations of regenerative medicine for the treatment of various pathologies, from organ injuries to chronic diseases and tumors.

Milan, March 4, 2013 – It has been almost seven years since 2012 Nobel Prize winner Shinya Yamanaka showed that skin cells could be reprogrammed in the lab to become very similar to embryonic stem cells. The discovery of induced pluripotent stem (iPS) cells is predicted to revolutionize modern medicine: it will be possible to regenerate damaged tissue starting from iPSC reprogrammed from the patient's own skin cells, thus avoiding their rejection after transplantion. But still, many questions remain open today: how can we assess the safety and the functionality of iPS cells to be used to generate tissues, and someday even organs? At what stage of development can we already today use IPS cells to study human diseases *in vitro* and thus move toward addressing the uniqueness of each patient? To address these questions we first need to understand the molecular mechanisms underlying the full reprogramming of differentiated cells. It is this last question that Casola and Testa have answered in a study that appears today in the scientific journal *PLoS Genetics*.

Cellular reprogramming in the laboratory consists of repressing specific genes that enable differentiated cells to execute their specialized functions, and taking them back to the pluripotent stem cell stage, from which they can assume a new identity. Doing this requires the complete reset of the genetic program including the activation of genes required for the acquisition of a new specialization. In fact, only a small fraction of the approximately 25,000 human genes is expressed in a given cell. It is precisely the combination of genes turned on and off that determines each cell's identity, constituting its imprint, or "identity card". The mechanisms that regulate cell differentiation, and consequently also reprogramming, are dependent on proteins that act as molecular switches to control the activity of thousands of genes. One of these switches is **Polycomb**, a group of proteins that acts silencing over 6000 genes, and whose involvement in the formation of tumors has been amply demonstrated. Defects in the regulation of Polycomb function are common in many pathologies, from tumors such as lymphoma, prostate, brain and breast cancer, to genetic diseases such as Kabuki syndrome and some forms of mental retardation.

The news that emerges from the study published by Casola and Testa is that, in addition to playing a leading role in differentiation and tumorigenesis, Polycomb is absolutely indispensable in the process of cellular reprogramming. In fact, by inactivating thousands of genes simultaneously, Polycomb functions as a cellular identity switch, "allowing" the transition from a differentiated cell to a pluripotent stem cell. "Using a genome-wide functional analysis, we found that not all of the approximately 6000 genes regulated by Polycomb are equally important in the reprogramming process. Only a core subset must be turned off to ensure that a mature cell returns to an undifferentiated state. It is not possible to generate stem cells from skin cells without turning off these key genes (a kind of 'core business of the cell')" says Casola, director of the *Molecular Immunology and Biology of Lymphoma* research program at IFOM.

"This discovery is an important contribution to stem cell research. However, its implications are not limited to regenerative medicine." emphasizes Testa, who coordinates the *Epigenetics of Stem Cells* unit at the IEO, "Now that we have identified the key genes controlled by Polycomb we can study their functions under pathologically relevant conditions, such as in tumors. In light of our results, it is becoming increasingly apparent that cancer is the result of a disorder of cellular differentiation, in which the cell loses its identity and acquires new properties in a manner very similar to reprogramming."

Many of the mechanisms that lead a differentiated cell to become a stem cell orchestrate also the transition from a normal cell to a tumor cell, with striking similarities between the two processes. "This confirms, once again, the transversal value of basic research that using a multidisciplinary approach based on genomics, proteomics and computational biology has contributed to new knowledge in different areas of life sciences, in this case regenerative medicine and oncology", the scientists reflect. "Moreover, considering that the expectations surrounding these issues are very high, often beyond the real state of the art, advancements in basic research are fundamental, indispensable for building a solid and reliable knowledge framework".

The research was made possible thanks to the support from, among others, Epigen, ERC, AIRC, the Giovanni Armenise/Harvard Foundation and the "Young Scientists" award from the Italian Ministry of Labour, Health and Social Policies, which in 2008 selected and financed more than 50 research projects for scientists under age 40.

FOCUS: Polycomb, epigenetics and reprogramming

All the cells in our body, whether in the brain, heart, muscles or any other organ, have the same identical genome, yet it is clear that they differ in both form and function. What makes a neuron so different from a skin cell? Of the 25,000 genes in each cell, only a specific group is expressed in a given cell type, while the others are silenced. This selective mechanism occurs at the **epigenetic** level (from Greek meaning literally "above genes"), through chemical modifications that govern gene expression by activating or repressing certain genes, without altering the DNA sequence. One mechanism of epigenetic regulation is modification of histones, proteins that bind DNA and allow the formation of chromatin. Several types of histone modification have been identified, to date: methylation, ubiquitination, acetylation and phosphorylation. Taken together, these constitute a sort of "key" for reading the DNA, assigning an on or off attribute to each gene. Various laboratories around the world are working to decipher the epigenetic code, and many results have already been obtained, attributing functional significance to the different chemical modifications identified. One of these is methylation of the lysine at position 27 in histone H3 (indicated as **H3K27me**), which is read as a signal for **silencing**.

During embryonic development, when pluripotent stem cells differentiate to acquire the appropriate shape and function for the tissue they will form, a series of epigenetic modifications occur at the level of histones to activate the genes for specific cellular functions and to turn the others off. Since 2006, it has been possible to induce the reverse process in the laboratory: by changing the expression of specific genes, a fully differentiated somatic cell can be brought back in time to reacquire the characteristics of embryonic stem cells. This process, known as "reprogramming", has opened a new trajectory in the field of regenerative medicine. iPS cells have been studied for some time now, to determine their characteristics, assess their potential and, above all, to understand the differences and similarities between them and embryonic stem cells. From the epigenetic point of view, it is clear that the H3K27me silencing signal is fundamental for defining the identity of these cells, to the point that it constitutes the imprint that distinguishes the reprogrammed stem cells from somatic cells.

How is the H3K27me signal applied to histones? By means of the Polycomb complex of proteins, identified for the first time in the 70s in the fruitfly *Drosophila melanogaster*. Involved in cell differentiation and tumor transformation, the Polycomb complex was recently implicated also for a possible role in the reprogramming process. Using an innovative approach that integrates *in vivo* and *in vitro* studies with functional genomics, proteomics and computational biology, scientists at IFOM and IEO have discovered that the Polycomb complex acts as an identity switch. Simultaneously repressing the expression of over 6000 genes, Polycomb erases the imprint of somatic cells and allows the reacquisition of a stem cell identity. By investigating this "molecular switch" in a system where the change of identity is as obvious and profound as switching from a somatic cell to a stem cell, the researchers were able to do what had been impossible with other systems: to understand how this transition occurs at the molecular level. It emerges from their studies that only a subset of the genes silenced by Polycomb is actually necessary to ensure reprogramming: these genes constitute the founding core from which the whole process starts. The researchers themselves describe this group of genes as the Polycomb "core business" and indicate that it will be the starting point for future studies.

Alterations of the Polycomb axis are frequently associated with pathological conditions in humans. From cancer to genetic diseases, from embryonic malformations to mental retardation, many diseases are characterized by a strong imbalance in the level of H3K27me signals on histones. The explanation for this makes intuitive sense: Polycomb is an epigenetic factor that controls the expression of thousands of genes, thus when this master switch is defective, the effect on the cell is inevitably dramatic. The knowledge acquired from this study has important implications not only for regenerative medicine, but also for all areas in which the devastating effects of Polycomb alterations are being investigated: identification of the "core business" will guide new research, focusing it on a limited set of targets.