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# Back on Track: New Perspectives on Cancer Cell Reprogramming

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**Abstract**

The possibility to obtain induced pluripotent stem cells (iPSCs) starting from cancer cells has revealed itself to be a major step forward in the understanding of the mechanisms able to regulate stemness, differentiation and neoplastic transformation. Our work proposes a new differentiation method using rapamycin and an amorphous bone matrix to promote the commitment of neuroblastoma cells towards the osteogenic lineage, switching to a different germ layer, without an intermediate iPSCs step. We followed the process from a morphological point of view with immunofluorescence analysis, cytochemistry and electron microscopy and from a metabolic point of view with enzyme activity tests and protein expression analysis. We believe that the morphological and metabolic changes observed are the foundations for a new type of cancer cell reprogramming.

**Keywords:** Neuroblastoma; Cancer cell differentiation; Rapamycin; Scaffolds; Metabolic reprogramming

## Commentary

One of the greatest challenges cancer researchers face is unraveling the complex network of cellular signals and events that leads to cancer onset and progression. By approaching the study of cancer from different angles and viewpoints, we can expect to deepen our understanding and paint a richer picture of this network. Progress has been made by reprogramming cancer cells into iPSCs to then differentiate them into various cell types, however we were intrigued by the possibility to reach this end result more directly. The paper "differentiation of human neuroblastoma cells toward the osteogenic lineage by mTOR inhibitor" [1] demonstrates the possibility to directly differentiate a human neuroblastoma cell line (SH-SY5Y) into osteoblast-like cells, without the intermediate step of forming iPSCs by inserting transcription factors [2]. Given this evidence, can we hope that another type of "reprogramming" is possible?

It is well known that a reprogramming of the healthy cell is observed during tumorigenesis, led by genetic mutations and/or physiological disturbances that deregulate basic processes in cellular homeostasis and, often also thanks to the tumor microenvironment, allow the acquisition of "stemness" features. This greater "stemness" leads to a particularly aggressive cancer phenotype with a greater tendency to form metastases. What is shared by the entire cancer population is a phenomenon known as "metabolic reprogramming", during which the cells favour energy production through the glycolytic pathway even in normoxic conditions, at the expense of the oxidative phosphorylation pathway. This phenomenon together with lactate production is recognized as the Warburg effect [3,4].

We chose a human neuroblastoma cell line, SH-SY5Y, which is a highly aggressive and undifferentiated neuroendocrine cancer type derived from the neural crest. It provided us with a good and appropriate model to test the possibility of forcing a cancer cell to differentiate into a distinct germ layer sheet with respect to its origins. The aspects considered in this paper are both morphological and metabolic changes that occur during the differentiation process of the cancer cell towards a new found normalcy, at the end of which most of the acquired malignancy characteristics are not being expressed anymore.

Our osteogenic differentiation protocol is based on rapamycin, already used for the differentiation of embryonic stem cells [5], combined with a second fundamental component: bone matrix granules acting as a scaffold to promote the differentiation process towards the osteogenic lineage. These matrix granules mimic the natural microenvironment in which osteoblasts act and physiologically reside. The key role of these granules is confirmed by SEM analysis, thanks to which we can observe osteoblast-like cells adhere to the scaffold. Multiple imaging techniques also confirmed the production of inorganic calcium phosphates and secretion of osteocalcin a typical osteogenesis marker.

Another important aspect highlighted in this article is the metabolic reorganization (glycolytic to oxidative) that occurs in differentiated cells under the guide of important transcription factors such as Myc and p53 [6]. In our cells we observe a change in protein expression characterized by a disappearance of Myc expression and a downregulation of p53. This metabolic switch is evidenced by a modulation in the expression of glycolysis enzymes, with a lowered expression of the glycolytic enzyme isoforms involved in the Warburg effect such as HK2, IDH2 and PKM2 [7] Concomitantly, we observe an increase in oxidative phosphorylation, as deduced by the decreased ATP content in differentiated cells compared to SH-SY5Y cells, following the *in vitro* inhibition of the mitochondrial electron transport chain. Restoration of mitochondrial activity is accompanied by a marked increase in Sirt3 activity [8].

This paper presents an alternative approach to cancer cell reprogramming towards a differentiated state, by skipping the iPSCs production passage usually found in the literature [9]. We supposed that cancer cells already showed sufficient stem-cell-like behavior to be ready for direct differentiation towards other germ layer lineages. In this context we were able to avoid problems correlated with iPSCs generation such as variable induction efficiency and culture time,

depending on the methodology used [10,11]. Our data suggest that this simplified approach is viable, at least for the SH-SY5Y neuroblastoma cell line (Figure 1).

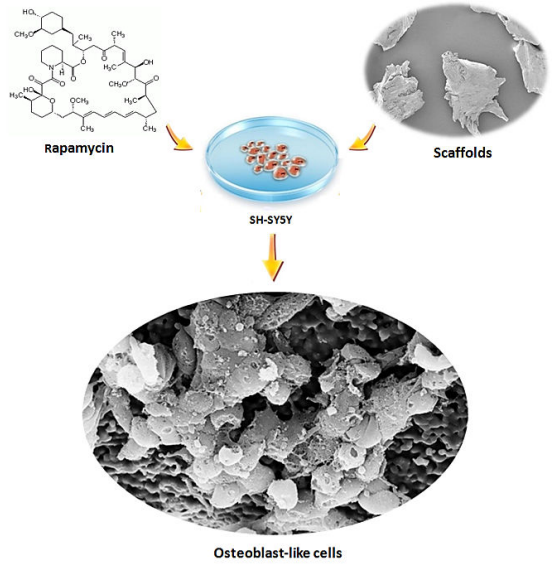


**Figure 1:** A representative scheme of the main actors involved in our differentiation protocol. Neuroblastoma cells are seeded with Rapamycin and scaffolds for twenty days, during this period the cells undergo metabolic changes and morphological modifications that are appreciable thanks to SEM imaging.

The guiding concept of this work is to consider the cancer cell population as a collection of cells that present a heterogenous mix of phenotypes, with varied degrees of differentiation, proliferation index and invasivity. Keeping this in mind, one could view the neoplastic transformation as a form of faulty deprogramming of the adult cell. If this were true, the right perspective might not be one of cellular destruction but rather of "reeducation", turning the evolutionary advantages acquired by the cancer cell to our favour by identifying the right stimuli to do so.

In conclusion, it appears clear that there are still multiple aspects of metabolic regulation and cellular differentiation that require further exploration. We believe that this novel protocol has the potential to change our approach to cancer reprogramming and provides a model to decipher the metabolic switch underlying it. It would be of interest to verify our hypothesis on other types of cancer cells as well, and we hope that this reprogramming strategy could open up a new scenario to aid us in better understanding the mechanisms behind the neoplastic transformation.

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