

Quality control and characterization of normal and transformed rat mesenchymal stem cells

INTRODUCTION

Bone marrow derived mesenchymal stem cells (MSCs) are multi-potent precursor cells of non-hematopoietic stromal tissues with a high potential for self-renewal and differentiation. The excellent proliferation capacity of MSCs makes them attractive for the generation of large cell numbers for autologous stem cell therapy. Thus, several clinical and preclinical studies have been carried out and indicated that MSCs are a highly promising source for regenerative medicine. However, it has been shown recently that long-term cultured MSCs might exhibit undesirable immortalization, spontaneous transformation and tumorigenic potential in human and mouse.

Isolated rat MSCs that underwent transformation in early passages, so-called transformed MSCs (tMSCs), were compared with normal rat MSCs (nMSCs). The aim was to characterize transformed cell metabolic features such as cellular acidification, respiration and cell impedance/adhesion using the **Bionas Discovery™ 2500 system**.

MATERIALS & METHODS

Cell culture. MSCs were isolated from bone marrow of young Lewis rats according to standard protocols and cultured at 37 °C and 5% CO₂ until passage 3. Cells showing an abnormal morphology were isolated and cultured separately.

Normal MSCs and sub-colony forming tMSCs (Fig. 1) were seeded onto the **Bionas Discovery™ metabolic chip SC 1000** (Fig. 2) at a density of 3x10⁴ cells/chip.

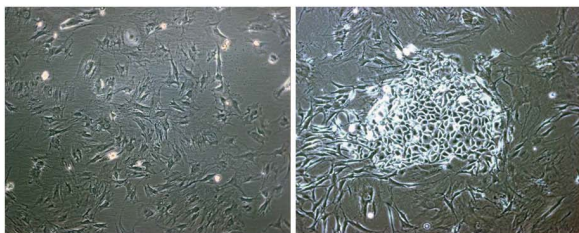


Fig. 1: Morphology of normal MSCs at day 10 in culture (left) and transformed MSCs at passage 3 (right) forming a subcolony with different morphology and proliferation behaviour (from Furlani et al. 2009).

Measurement procedure.

Supply of fresh medium was achieved by fluidic perfusion (56µl/min) following a stop and go cycle of 4 minutes each. Changes of bioenergetics of tMSCs and nMSCs were detected during the stop phase. After the detection of baselines for

acidification, oxygen consumption and cell impedance/adhesion signals of metabolic activity for both cell types were measured for 33 hours. Acidification and respiration rates were calculated as a slope of the curve during the stop phase.

To end the experiments, cells were killed by adding 0.2% Triton X-100 to get a neutral signal without any living cells.

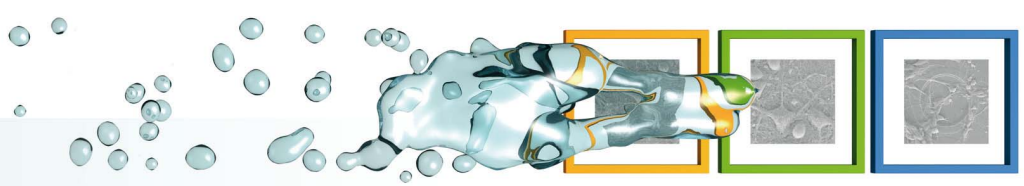


Fig. 2: Area of the chip showing all three types of sensors covered with transformed MSCs

Analysis in the **Bionas Discovery™ 2500 system**.

In comparison to nMSCs, tMSCs showed distinctly higher acidification rates. This increased acidification correlated with an increase of oxygen consumption (Fig. 3).

However, transformed MSCs showed a lower impedance signal than nMSCs. In contrast to nMSCs, tMSCs remained stable throughout the entire experiment.



COMPARISON OF nMSCs AND tMSCs

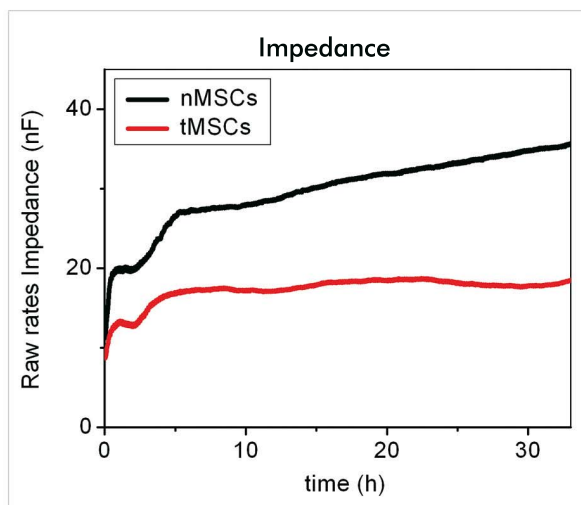
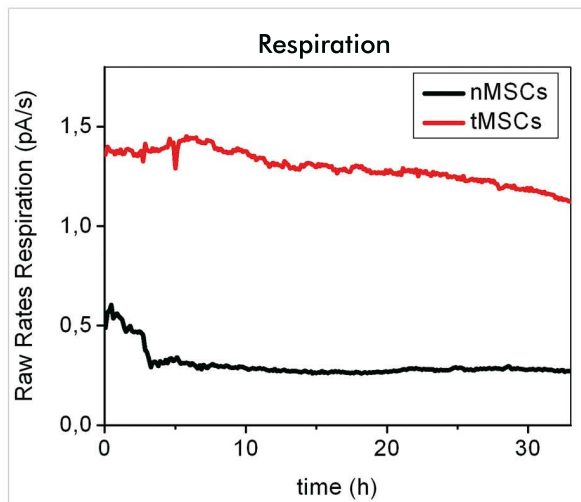
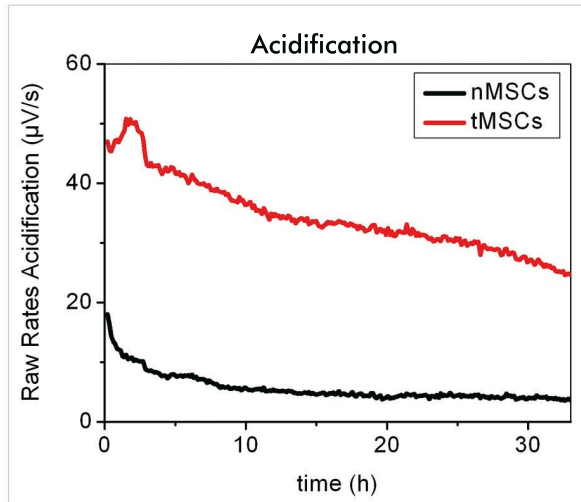


Fig. 3: Raw rates of the acidification, oxygen consumption (respiration) and cell impedance/adhesion of normal (black) MSCs and transformed (red) MSCs (from Furlani et al. 2009).

CONCLUSION

Under standard culture conditions, bone marrow-derived rat MSCs may undergo spontaneous transformation in early passages. An analysis of the metabolism comparing the raw data of tMSCs to nMSCs showed elevated acidification and respiration rates of tMSCs, while the impedance/adhesion rate was lower than that of nMSCs and remained stable over time.

Standardization of the quality of cells is of major importance for quality control procedures prior to cell transplantation for regenerative medicine. In this work we could detect differences in the metabolic activity of tMSCs versus nMSCs with the **Bionas Discovery™ 2500 system**. This metabolic characterization would be highly recommended before the administration of human MSCs to patients in pre-clinical and clinical trials for cardiac regeneration after ischemic cardiovascular diseases. The **Bionas Discovery™ 2500 system** supports the establishment of standardized protocols to ensure the safe usage of MSCs in regenerative therapies and their full clinical therapeutic benefit.

KEYWORDS

Metabolic activity, mesenchymal stem cells, transformed mesenchymal stem cells, quality control, regenerative medicine

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LITERATURE

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