

Correlating cell architecture with osteogenesis: First Steps towards live single cell monitoring

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INTRODUCTION: Cell shape and regulation of biological processes such as proliferation and differentiation are to a large degree connected^{1,2}. For instance it has been demonstrated that cell spreading increases osteoblast differentiation in pre-osteoblastic progenitors³. Human mesenchymal stem cells (hMSCs) which were allowed to adhere, flatten and spread differentiated into osteoblasts while unspread and round cells showed differentiation towards adipocytes⁴. Investigation of the possible relationship between cell shape and function is therefore important for tissue engineering as well as development of cell based sensors.

Cell spreading requires a firm contact with the underlying substrate, with focal contacts (FC) being the primary sites of adhesion. They consist of a large number of clustered transmembrane proteins (integrins). FC integrins connect the cell cytoskeleton with the cell substratum. The gradual process of osteogenesis can be followed by different proteins being expressed at various time points, comprising early (e.g. *runx2*) and late (e.g. *osteocalcin*) genes. The aim of our project is to correlate the cell architecture with osteogenesis in single cells.

METHODS: We used gene constructs containing the genetic information for the focal adhesion proteins vinculin and paxillin (pEGFP-vinculin, pGFP-paxillin) fused to green or red fluorescence for nucleofection of human bone marrow cells. This method allows fluorescently tagged functional proteins to be visualised and monitored during FC formation and disintegration. Nucleofection allows the delivery of the gene of interest directly into the nucleus and cellular distribution of fluorescence was verified after 1-4 days. In addition cells were transfected with a gene construct reporting for osteocalcin gene activity.

RESULTS: Cells transfected with the fluorescent-labeled vinculin or paxillin showed the expected accumulation of green

fluorescence signal at focal adhesion sites. The correct localisation of the vinculin was confirmed by staining against endogenous vinculin, suggesting that the tagged protein is correctly synthesized. GFP expression regulated by the osteocalcin promoter could be detected in osteoblastic cells.

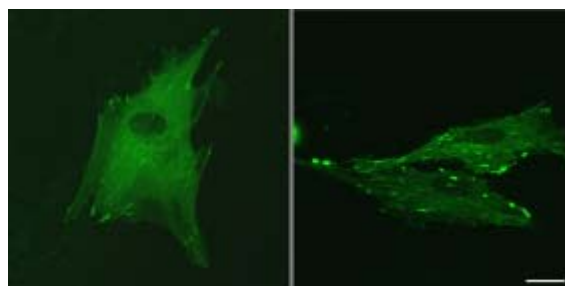


Fig. 1: Transfection of human bone marrow cells with proteins paxillin (left) and vinculin (right) fused to green fluorescent protein. [scale bar 20 μ m] Cells showed the expected accumulation of fluorescence in focal adhesion sites.

DISCUSSION & CONCLUSIONS: Our primary results suggest that transfection of human cells with the present fluorescent-labelled adhesion proteins is efficient and therefore qualified for live cell monitoring.

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