Methylation Patterns as a Marker for Tracking of Autologous Transplanted Stem Cells

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Introduction
Regenerative medicine and especially the use of stem cells is a rapidly advancing field in medical research and applications. In most cases, mesenchymal human stem cells (MSCs) are used as both cell suspension or as a part of tissue engineered products and therefore must undergo "substantial manipulation" such as cultivation or expansion. In vitro manipulation of cells is often accompanied by the occurrence of genetic aberrations, possibly leading to malignant transformation and tumorigenesis after transplantation. Therefore it is important to identify the origin of the cells triggering tumor formation. As long as the recipient receives cells, tissues or organs from a genetically non-identical donor (allotransplantation), standard fingerprinting methods can be applied. If, however, the patients' own cells are manipulated in vitro and transplanted (autologous transplantation), they cannot be identified after transplantation. Therefore it is necessary to label autologous transplanted stem cells before transplantation. In this project we establish a new approach to facilitate cell identification: specific epigenetic patterns of DNA fragments (HLA promoter regions) as a distinctive endogenous cell signature of substantially manipulated MSCs.

Results and Outlook
As a first step promoter regions of HLA genes were successfully identified using GeneFinder (hidden markov model). Isolated mesenchymal stem cells were incubated in growth and differentiation media, both with and without the addition of the inflammation factor IFN-γ. Both DNA and RNA were extracted from all four different cell samples. Furthermore the first steps of methylation pattern analysis were carried out via sequencing before bisulfite conversion. Next, bisulfite converted promotor regions will be sequenced and compared with the original sequence in order to determine the methylation patterns and their stability.

Future Perspective
Due to this work we will be able to show the influence of the inflammation factor and the differentiation state towards a change in methylation patterns. In case of stable methylation patterns an artificial methylation pattern will be introduced into MSCs. Subsequently the influence of multiple passages on pattern stability will be analyzed.

This project aims to establish a new system of labeling and tracking autologous transplanted stem cells using artificial methylation patterns.

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Figure 1: Overview of applied Bisulfite Conversion of the EZ DNA Methylation-Lightning™ Kit (Zymo Research)