Redox state surveillance in human fibroblast cultures derived from circulating progenitor cells (CPCs)

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Progenitor cells with clonogenic properties have been detected in peripheral blood. In this study we have tested the possibility to obtain, directly from peripheral blood, circulating progenitor cells (CPCs). CPCs play a role in cellular replace in the body. We perform an easy protocol to maintain in vitro CPCs useful for toxic assay. In this work we (i) show how to expand circulating CPCs and pro-differentiated them in cells with fibroblast cellular phenotype; (ii) provide three cell culture systems with different proliferation rate; (iii) provide a correlation between intracellular redox and in vitro proliferative state. The procedure was applied on health voluntaries (median age of 43 years) enrolled by specific inclusion criteria and characterize by a Glutathione Reduced (GSH) level of 25-41 mg/100ml (measured in whole blood). Growing evidence show the impact of biological rhythms on the traffic of hematopoietic stem cells (HSCs) and their proliferation and differentiation capacities. Recent evidence supports the role of the sympathetic nervous system in HSCs behaviour's regulation, both directly and through supporting stromal cells. An examination of healthy donors who were contributing HSCs for bone marrow transplantation revealed that the average yield was greater for those who underwent the procedure in the afternoon compared with those who were harvested in the morning. The maximum release of HSCs at the beginning of the rest period for both species (early night for humans, early morning for mice) supports the intriguing possibility that this phenomenon may contribute to regeneration. On these bases we have collected blood samples in day's different moment and correlated the moment of sampling and correspondent *in vitro* cell adhesion rate.
Phenotypic characterization of cultivated cells obtained, showed the presence of CD34/CD133 subset (max percentage founded 1,9%). We have compared proliferative rate in normal adherent cells and adherent cells derived from tumour subjects with significant differences ($p = 0.0005$ Student t-test). Profile characterization had displayed an 11% (average value) of colonies of adherent fibroblast with phenotype CD45 negative and Fibronectin positive expression ($CD45^{neg}$/$Fibronectin^{pos}$). The GSH level showed fluctuations correlated with the proliferation state of cultivated cell system. In particular, the levels of glutathione have a parallel trend with the cell cycle phases especially S/G2M (Pearson's correlation coefficient was of 0.90; $p<0.05$). Increasing S/G2M phases we have a significant glutathione levels reduction, as reported by other studies. Moreover, oxidizing environments have resulted in apoptosis or necrosis in manner dose dependent. In conclusion, we have identified a novel cellular model to monitor *in vitro* redox environment changes that can occur in human cells derived with an easy 'Blood Sampling'.