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Analysis of clonal diversity and clonal size in an *in vivo* model of multidrug resistance 1 (MDR1) gene transfer to human peripheral blood progenitor cells

Geboren am 25.02.1976 in Oradea (Rumänien)
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Mobilized human PBPCs or human cord blood cells were transduced with two different retroviral vectors and then transplanted into NOD/SCID mice to study the human hematopoiesis in this xenotransplantation assay. Two methods were used to follow the progeny of transduced individual cells. Ligation-mediated PCR method allowed the identification of individual cell clones, while RQ-PCR was used to quantify some individual cell clones.

Up to 23 repopulating human cell clones were found 6-8 weeks after transplantation in one mouse, which was higher than previously detected by Southern blot analysis. The theoretical number of transduced clones contributing to hematopoiesis was estimated to be 168. Characterizing vector integrations on genomic level the oncoretroviral vector SF91m3 integrated with significantly higher frequency into chromosomes 17 and 19, while the lentiviral vector RRLsin18 showed an insertional preference for chromosome 11.

To quantify the clones originated from individual retrovirally transduced progenitors transplanted into NOD/SCID mice the plasmid library of junctions between proviral and human genomic DNA generated during the LM-PCR analyses was used for standard plasmid dilutions as a reference. Individual cell clones, comprising 0.01-1.82% of all transduced cells could be identified. It was estimated that 56-10000 clones would make up the transduced cell population, thus a polyclonality of human hematopoiesis was demonstrated in the NOD/SCID mouse transplantation model. The real number of simultaneously active clones ranges between these estimates.

The methods presented here allow the study of human hematopoiesis at the level of single repopulating cells and the results suggest that a plethora of individual hematopoietic cells are simultaneously contributing to human hematopoietic BM engraftment in xenograft models.