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RESEARCH ARTICLE

Expression of the miR-221/222 cluster can safeguards transplanted HSC from loss of lymphoid-myeloid multipotency

Peter K. Jani[1], Fritz Melchers[1*]

[1]Deutsches Rheuma-Forschungszentrum, Berlin

[*]fritz.melchers@unibas.ch

ABSTRACT

A microRNA cluster, miR-221/222, is expressed in quiescent and activated HSC of mice. The expressions of fos and jun, hence AP-1, and a collection of other immediate early genes (IEG), are up-regulated in miR-221/222-deficient HSC. Social stress and HSC transplantation induces comparable up-regulation of IEGs. Stress, as well as miR-221/222-deficiency leads to decreases in the numbers of quiescent HSC and increases in the number of activated hematopoietic (MPP2-4) progenitors, as well as numbers of progenitors of emergency granulopoiesis. Serial transplantations of miR-221/222-deficient HSC generated HSC in the recipient bone marrow, prematurely lose their lymphoid repopulation capacities – normally a sign of aged HSC. Consequently, enforced expression of miR-221/222 should preserve and enhance lymphoid potency of HSC. These results suggest that the expression of miR-221/222 genes themselves and target genes of miR-221/222 in HSC should improve HSC performance in bone marrow niches and their lymphoid development in the transplanted host. Since the miR-221 and -222 genes are also expressed in human HSC, it is likely, that these new targets should be applicable to the improvement of human bone marrow transplantation.

Introduction

In human autologous and allogeneic hematopoietic stem cell (HSC) transplantations (ASCT), rapid and functionally efficient establishment of the innate and the adaptive immune system of the donor in the transplanted recipient is important for the recovery and long-term survival of the patient[1-3].

Current protocols of autologous and allogenic HSC transplantations rely on long-term liquid-nitrogen-stored frozen pools of cells. Usually, human HSC are enriched from blood after mobilization of bone marrow cells using G-CSF or cyclophosphamid treatment. Even other sources, not necessarily only stem cell-enriched pools of cells are used for transplantations. At the day of transplantation, cells are washed from freezing media and injected intravenously to recipients in high concentrations to reach sufficient numbers of reconstitution-potent stem cells for efficient engraftment. All procedures collecting and handling donor HSC "ex vivo" cannot avoid cellular stress[4-6]. Several attempts have tried to increase the protocols for a better engraftment efficiency and faster, more complete lineage reconstitution. Some of these trials are discussed. We propose two alternative approaches, in which external sources of microRNA-221 and -222 are administered "in vitro", immediately before transplantation, in order to increase the efficacy of ASCT by suppressing stress-mediated stem cell activation and differentiation to emergency granulopoiesis[7].

Dependent on the quality of the source and the numbers of human HSC from peripheral blood (PBSC), from bone marrow (BM) or from umbilical cord (UBC), neutrophils appear reconstituted within two to four weeks, natural killer (NK) cells within one to three months and T cells within three months, while the reconstitution of B cells can take one to two years[1]. This slow reconstitution, especially of the adaptive immune system of T and B cells, leaves the transplanted recipient for weeks and months susceptible to life-threatening viral, bacterial and fungal infections. It is hoped, that accelerated, fully differentiated reconstitution of the innate and the adaptive lymphoid donor cells in the transplanted hosts could improve their short-term immune defenses against infections and their long-term functioning in the diverse functions of the immune system. With aging, the capacities of HSC to differentiate to lymphoid cells, i.e. to T- and B-lymphocytes of the adaptive immune system decreases, while differentiation to myeloid cells, including granulocytes, appears to remain intact[7]. Hence, transplantation of HSC from young donors is preferable to old donors.

Strategies to improve immune reconstitution by stimulating progenitor cells during development of the transplanted HSC and their progeny have been proposed[3]. They include IL7[8,9] and flt3-ligand[10-12] as stimulators of early lymphoid progenitors including common lymphoid progenitors (CLP).

Our recent studies in experimental mice have identified a microRNA cluster, miR-221/222, expressed in quiescent and activated HSC, and genes, whose expression are regulated by this miR-221/222 cluster as new targets to improve HSC performance in bone marrow niches and their lymphoid development in the transplanted host[13]. The miR-221 and -222 genes are also expressed in human HSC. This

makes it likely, that these new targets should be applicable to the improvement of human bone marrow transplantations[14].

Results and methodology

HSC reside in cellular niches in bone marrow. Some of them remain quiescent for the entire life. These quiescent HSC are capable to long-term reconstitute bone marrow progenitors as well as all differentiated lineages of hematopoietic cells, i.e. erythroid, megakaryocytic, myeloid and lymphoid lineage cells. Two other populations of HSC appear activated, either to enter cell cycle or to enter differentiation to selective granulopoiesis without initial proliferation (**Figure 1**). They have severely reduced or no capacities to long-term repopulate the hematopoietic progenitors and differentiated lineages upon transplantation. All three states of HSC express miR-221/222. Stress, exerted on HSC either "in vivo" (e,g, by infections,[15]) or "in vitro" (e.g. by handling of HSC during isolation and preparation for transplantation, [10-12]) decreases the numbers of quiescent HSC and increase the number of proliferating progenitors as well as cells differentiating to emergency granulopoiesis.

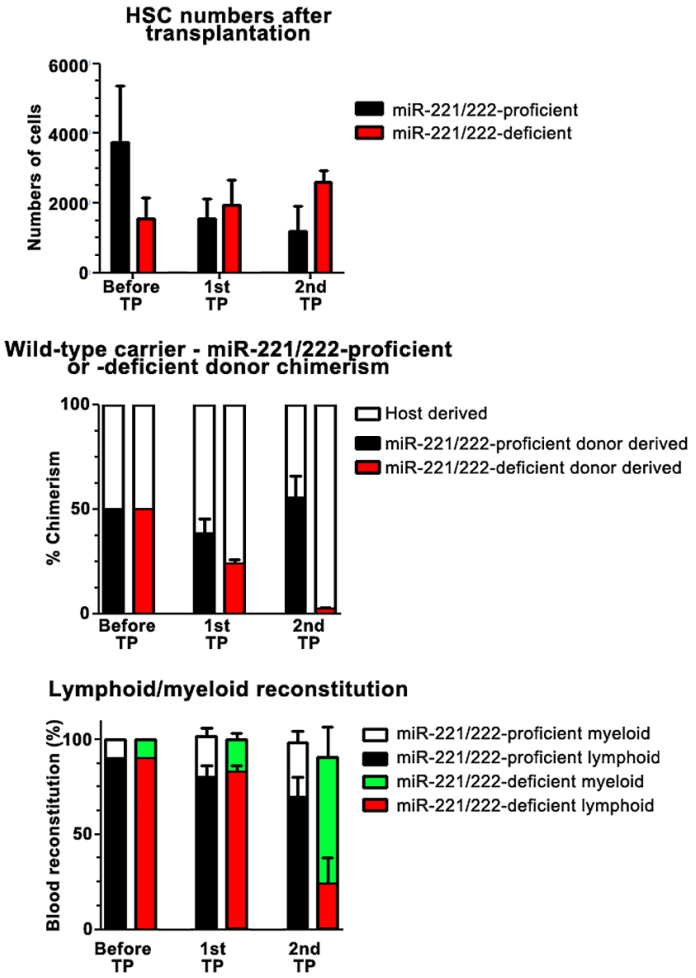

Figure 1: Serial transplantations of miR-221/222-proficient and deficient mouse HSC. Reconstitution capacity and lymphoid-myeloid multipotency of miR-221/222-proficient and –deficient murine HSC tested in competitive transplantations.

The three states of HSC are characterized by the selective upregulation of expression of genes, which function selectively in one of these three HSC populations[13]. Thus, HSC activated to cell cycle – among others – have selectively upregulated genes known to be expressed either in the G1/S or the G2/M phases of the cell cycle. HSC activated by either stress or miR-221/222-deficiency to granulopoiesis, have immediate early response genes (IEG) selectively upregulated.

Among the IEG, we have identified fos, the partner of jun in the AP-1 transcription factor complex as a direct target of miR-221/222 action, and another five to ten genes as indirect miR-221/222 targets. Thus, miR-221/222 expression in HSC is expected to result in a lower expression of fos/AP-1, a lower expression of the indirect target genes, and consequently, in a higher proportion of quiescent HSC and a lower proportion of stress-induced HSC differentiating to granulocytes.

In line with these expectations we found, that miR-221/222-deficiency, induced by targeted disruption of this locus in mice, activates HSC from their quiescent state and induces, upon transplantation, decreased lymphoid differentiation, while retaining myeloid-granulocytic differentiation potential. With increasing age, HSC lose their capacity to differentiate to lymphoid cells[16]. Hence, the miR-221/222-deficiency appears to prematurely "age" HSC. We find, that serial transplantations of miR221/222-deficient HSC generate normal numbers of BM-homing HSC, but these HSC lose their multipotency to develop lymphoid cells much more rapidly than their miR221/222-proficient counterparts (**Figure 2**)[13]. Consequently, we expect enhanced miR221/222-expression in transplanted HSC to reduce the stress-induced loss of lymphoid differentiation capacity, and to maintain, or even increase the numbers of HSC in quiescence and longevity, with their and multipotent lymphoid/myeloid differentiation capacity. MiR-221/222 expression should keep HSC "young" or rejuvenate them for better lymphoid reconstitution of the transplanted host after stressful bone marrow transplantation.

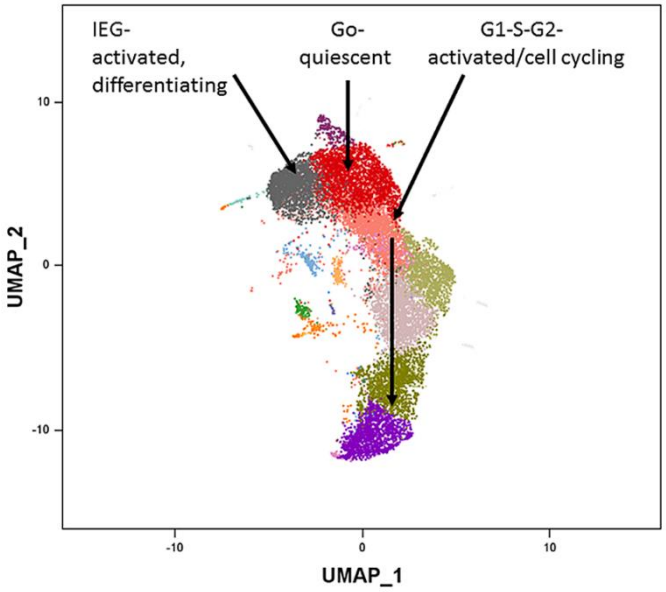

Figure 2: Three clusters of HSC in mouse bone marrow. Single cell deep RNA sequencing analyses of LSK CD34$^{+/-}$ CD150$^{+/-}$ CD48$^{+/-}$ HSC, MPP1 and MPP2 cells from mouse bone marrow detect one cluster of quiescent HSC (red), one cluster of activated, immediate early genes (IEG)-expressing, non-proliferating, granulocyte-directed differentiating HSC (dark gray), and five clusters of activated HSC, one for activated (pink), and four for cycling, proliferating HSC, thus expressing genes of the different G1-S-G2-M phases of the cell cycle (light green, light violet, green, violet).

Discussion

To introduce miR-221 and miR-222 into HSC during their "in vitro" preparation prior to transplantation, we suggest two possible ways for administration. One way would be to introduce expression-controllable vectors containing the miR-221 and miR-222 genes into HSC preparations by lentiviral[17] or adenoviral[18] transduction, similarly to the approaches, where such virus-based gene-therapy has been used in clinical applications. Our strategy would reduce the risk of viral transduction of irrespective cell types, because the vector would be strictly administered "in vitro" to stem cell-enriched pools of cells prior to transplantation. To control the limits of their activity, the expression of miR-221 and miR-222 should be controllable by reversibly inducible promotors, such as the doxycycline (Dox)-inducible promotors[19,20], which we have used in transplantations of miR221 into murine preB cells[21,22]. The usage of Dox would be in line with conventional guidelines on antibiotic treatment after ASCT.

In an alternative strategy, the degradation-protected, pseudo-U-containing[23] mature form of microRNA-221 and -222 could be delivered by lipofection[24]. In proposing this strategy, we assume that mature microRNAs can interact directly with target sequences-containing mRNAs (for miR-221/222: fos mRNA), and that pseudoU-containing microRNAs can do so as well as normal microRNAs to regulate mRNA's translation at various level. Again, the "in vitro" transfection of stem cell-enriched pools of cells prior to the autologous stem cell transplantation would have the clear benefit of a direct treatment of HSC. The dilution of the degradation-protected miRNA vector in proliferating lineage-progenitor cells would help to focus the stress-inhibiting, lymphoid differentiation-protecting actions of the miR-221 and-222 expression almost exclusively on early, quiescent, non proliferating HSC.

Conflicts of Interest Statement:

None to declare.

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None

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