Regulatory role of fibroblast growth factors on hematopoietic stem cells

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2007

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Download date: 01-01-2019
CHAPTER 5

Summarizing Discussion and Future Perspectives
Summarizing Discussion

Expansion and maintenance of self renewing primitive hematopoietic stem cells (HSCs) would have major implications in the areas of stem cell transplantation and gene therapy. The ultimate goal of many scientists is to successfully expand and maintain stem cells in their primary functional characteristic, namely their ability to engraft and sustain long-term hematopoiesis. Establishing the ideal in vitro growth conditions to expand and maintain HSCs has proven to be difficult. Recently, strong evidence is emerging indicating that the large family of fibroblast growth factors (FGFs) and FGF receptors (FGFRs) may play a key role in stem cell maintenance. Chapter 1 provides a conceptual overview on FGFs and their receptors in maintaining tissue homeostasis in HSCs, neural stem cells (NSCs) and embryonic stem (ES) cells, in order to retain the self renewal capacity of stem cells. Commonalities do exist between these three distinct stem cell systems. We summarize evidence that FGFs are growth factors crucial for the regulation and culturing of all three of these stem cell systems.

In Chapter 2 we studied the role of FGFs to maintain HSC function by culturing unfractionated bone marrow (BM) cells in serum-free media supplemented only with FGF-1, FGF-2 or the combination of both (FGF-1 + 2). Bone marrow cells were cultured for a total of five weeks and then competitively transplanted into lethally irradiated hosts. Cells cultured in FGF-1 + 2 showed a 5-fold and 1.5-fold increase in repopulating units after culturing for one and five weeks respectively. In addition, we co-cultured Lin- Sca-1^+ c-Kit^+ (LSK) with unfractionated BM cells for a total of five weeks in FGF-1 + 2 and transplanted without competitors into lethally irradiated recipients. Overall, the data demonstrated that FGF cultured BM cells can be maintained for up to five weeks while retaining long-term repopulating ability (LTRA) and that all FGF-induced stem cell activity was derived from the LSK population. Furthermore, the data signifies the importance of the stem cell niche, a specialized microenvironment that houses, regulates and protects stem cells. We could not culture purified LSK cells in serum-free medium supplemented only with FGF-1 and/or FGF-2. However in the presence of unfractionated BM cells, LSK cells proliferated, suggesting that FGFs may be acting on other cell types to induce stem cell activity in vitro. Receptors for FGF-1 have been shown to be present on primitive hematopoietic cell subsets. Therefore, it is also highly probable that FGFs maintain...
stem cells by acting on FGFRs expressed on the stem cells. Additionally, non-LSK cells present in the stem cell niche may carry FGFRs and therefore be responsive to FGFs, playing an important role in the maintenance of stem cells. We speculate that BM elements in the co-culture act as a pseudo niche facilitating the proliferation of stem cells in vitro. These results demonstrate that we can maintain stem cell in culture for up to five weeks with LTRA.

Quantitative PCR (QPCR) analysis in Chapter 3 demonstrated that FGF-1 and FGF-2 were predominantly expressed in LSK cells. The presence of both FGFRs and high expression levels of FGFs on LSK cells strongly implies that FGFs may regulate HSCs by autocrine signaling. To further examine the role of FGFs on HSCs, in Chapter 3 we retrovirally overexpressed FGF-1 and FGF-2 in 5-Fluorouracil (5-FU) treated BM cells. In addition, to assess the role of nucleocytoplasmic trafficking of FGFs in hematopoietic cells, two mutant isoforms which altered the phosphorylation status of FGF-1 were created and overexpressed. In the first mutant, the serine residue at phosphorylation site 130 was exchanged for glutamic acid (S130E). This mutant was expected to mimic the phosphorylated state of FGF-1, constitutively exporting FGF-1 to the cytoplasm. For the second mutant, the serine was exchanged for alanine (S130A) which was hypothesized to prevent FGF-1 phosphorylation and remain localized in the nucleus. Higher molecular weight isoforms of human FGF-2 (21-22, 22.5, 24 and 34kDa) contain an upstream nuclear localization signal (NLS) which translocates FGF-2 into the nucleus. The smaller 18kDa isoform is confined to the cytoplasm. To create a nuclear localized isoform of mouse FGF-2, an artificial NLS was inserted upstream of FGF-2 (NLS/FGF-2).

Fluorescent images of hematopoietic cells overexpressing wild-type (WT) FGF-1, S130E or WT FGF-2 retroviral vectors showed that both FGF-1 and FGF-2 were expressed predominantly in the cytoplasm. In contrast, overlay images of hematopoietic cells overexpressing S130A and NLS/FGF-2 revealed that 2% of FGF-1 positive cells and 11% of FGF-2 positive cells were localized in the nucleus respectively. Although FGF-1 and FGF-2 mutant proteins were not predominantly localized in the nucleus, compared to WT FGF-1, S130E and WT FGF-2 nuclear localization did increase. More sensitive methods such as cell fractionation studies should be performed to clarify these results. Recently, using cell fractionation studies, Wiedlocha et al. showed that WT FGF-1 was found in all fractions (membrane, cytoplasmic and nucleus) of the cell, S130A was found in the nuclear fraction.
whereas S130E was mainly in the cytosolic fraction. These data indicated that the translocation of FGFs appears to be a dynamic process and each FGF can be regulated differently.

Unfortunately, transplantation studies with WT FGF-1, WT FGF-2, S130E and NLS/FGF-2 overexpressing cells resulted in low donor chimerism levels in primary recipients and little to no reconstitution in secondary recipients. This was unexpected as previous studies from our group (as described in Chapter 2) had shown that the unfractionated BM cells treated with exogenously added FGF-1 and/or FGF-2 were capable of long-term repopulation. These results suggest that the constitutive overexpression of FGF-1 and FGF-2 does not increase the repopulating potential of hematopoietic cells. Interestingly, secondary recipients transplanted with S130A overexpressing cells showed a delayed but marked increase in donor chimerism levels and a significant increase in GFP+ cells. These results strongly suggest that nuclear localized FGF-1 may play an important role in maintaining stem cell quality.

In vivo competitive transplantation assay is the ‘gold standard’ to test whether BM derived cells are indeed HSCs with the potential for reconstituting all hematopoietic lineages. The competitive repopulation assay has two key features. The first is that it enables the detection of a very primitive class of hematopoietic stem cells and the survival of lethally irradiated mice transplanted with very low numbers of such cells. The second is the use of a limiting dilution experimental design to allow stem cell quantitation. To highlight the effectiveness of this assay, in Chapter 4 we use the competitive transplantation assay to compare the functional qualities of mobilized peripheral blood (MPB) stem cells to normal BM stem cells. Mobilized peripheral blood has been used for the past 10 years in place of BM as a source of stem cells for transplants. Their ease of collection and ability to promote faster regeneration of neutrophils and platelets makes them a primary source for HSC transplantation. Transplantation of MPB stem cells in competition with BM stem cells demonstrated that MPB stem cells have a reduced long term repopulation potential. This impairment in repopulation potential was due to the presence of fewer stem cells rather than a decrease in stem cell quality. In actual fact, secondary transplantation of MPB stem cells indicated that the quality of stem cells from MPB did not decrease after transplantation and that exhaustion of initially engrafted stem cells is similar for both
BM and MPB stem cells. Clearly, in a clinical setting, more blood stem cells must be transplanted to compensate for the decrease frequency of stem cells.

**Future Perspectives**

In this thesis the exogenous and endogenous effects of FGF-1 and FGF-2 were examined. We have shown that FGFs, in particular FGF-1 and FGF-2 play an important role in regulating and maintaining stem cells. In total, 22 FGFs exists (not including spliced forms) and we have shown that two out of 22 FGFs are able to maintain HSCs. Most stem cell studies carried out are restricted to FGF-1 or FGF-2. From mouse knock-out studies it has appeared that other FGFs are more potent. For example, deletion of FGF-4, FGF-8, FGF-9 and FGF-10 result in embryonic lethality whereas FGF-1 and FGF-2 knockout mice are viable and fertile. Given their pleiotropic effects, similar receptor binding properties, overlapping patterns of expression and sequence similarities, functionally redundancy is likely to occur. It will be interesting to assess the effects of other FGFs, in particular FGF-4, FGF-8, FGF-9 and FGF-10 on stem cells. Such studies would increase our understanding on the biological role of FGFs on HSC maintenance and regulation.

The mechanistic action of FGFs on stem cells remains unknown. Our results suggest that the intracellular function of nuclear localized FGF-1 is biologically significant. This indicates that the nuclear import/export trafficking pathway of FGFs may be key to understanding the mechanistic action of FGFs. Future studies should be aimed at assessing the trafficking pathway of FGFs in stem cells. Firstly, it will be interesting to determine which FGFs bind to which FGFR and to what affinity. It should be noted that a large number of splice variants of FGFR genes exist and must be taken into account. Secondly, it would be appealing to determine the stage of the cell cycle which enables the precise cueing of the nuclear localization of FGFs. This may provide valuable information as to how nuclear localized FGF-1 (S130A) maintained stem cell quality. Thirdly, it would be interesting to determine whether all FGFs, which are highly homologous, have the same trafficking pathway. The competitive transplantation assay serves as the only tool to detect long-term repopulating stem cells with the potential for reconstituting all hematopoietic lineages. We highlighted the effectiveness of this assay to study the qualities of MPB stem cells.
compared to normal BM stem cells. Blood cells mobilized with Granulocyte-Colony Stimulating Factor (G-CSF) have reduced repopulation ability due to a lower frequency of stem cells. Many growth factors capable of migrating stem cells from the BM to the blood exist. Each mobilizing agent, alone or in combination with chemotherapeutic agents affects the stem cell differently. It will therefore be interesting to examine the effects of different mobilization regimes and whether this will improve the stem cell frequency and repopulating ability of blood stem cells. This knowledge may be relevant and change techniques used in established clinical application.
Summarizing Discussion and Future Perspectives

References


