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Incorporation of humanized niche as a strategy for improving leukemic engraftment in immunodeficient mice

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SUMMARY

In vivo models of patient derived leukemias are utilized using immunodeficient mice that provide engraftment of xeno-transplanted leukemic cells without their rejection. Despite the achievement of considerable immunosuppression in the latest immunecompromised mouse, engraftment of patient samples is still not always possible or does not fully reflect the original disease characteristics. This points to the presence of inter-species differences in the hematopoietic niche. In order to improve engraftment options, a valid proposition is to model the human niche in mice. In recent years, this has been performed by adopting an osteogenic approach already used in bone-regenerative medicine and based on using a suitable biomaterial along with mesenchymal stromal cells capable of bone-formation. The review aims to present and discuss works describing leukemic engraftment in a hybrid-mouse model containing a humanized microenvironment and to point out important details and possible future directions of this research.

KEYWORDS

bone marrow, microenvironment, scaffold, biomaterial, leukemia, mesenchymal stromal cell

SOUHRN

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Inkorporace humanizované tkáně jako strategie pro zlepšení uchycení leukemie v imunodeficientní myši

Pro *in vivo* modely leukemií odvozených z klinických vzorků pacientů lze využít imunodeficientní myši, které umožňují uchycení leukemického štěpu bez jeho odvržení. Navzdory tomu, že bylo v imunokompromitovaných myších kmenech dosaženo značného potlačení imunity, není uchycení leukemických buněk možné u všech vzorků, nebo ve výsledku nemusí uchycené buňky odpovídat původní charakteristice nemoci v důsledku mezidruhových rozdílů v hematopoetickém prostředí. Možností, jak dosáhnout lepšího uchycení, je proto vytvořit ve zvířeti přímo lidské mikroprostředí. Toto bylo v několika posledních letech provedeno pomocí přístupu používaného pro tvorbu nové kosti, převzatého z regenerativní kostní medicíny, kde se využívá vhodného biomateriálu a mezenchymálních stromálních buněk schopných tvořit kostní tkáň. Záměrem této přehledové práce je představit a rozebrat studie popisující uchycení leukemie v modelu hybridní myši obsahující lidské mikroprostředí a rovněž poukázat na důležité detaily a další potencionální směřování tohoto výzkumu.

KLÍČOVÁ SLOVA

kostní dřeň, mikroprostředí, skelet, biomateriál, leukemie, mezenchymální stromální buňka

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INTRODUCTION

In vivo models of leukemias are an invaluable study tool, as they enable the best approximation of the situation in a patient's body and the testing of specific processes in a complex organism in contrast to the isolated conditions *in vitro*. Animal experiments may focus on observation of rather simple processes, e.g. engraftment of primary leukemic cells, or they may specifically focus on detailed investigation of specific targets and pathways, where the animals and/or injected cells are subject to complex modification, e.g. introduction of individual leukemogenic mutations into healthy hematopoietic stem cells (HSC) or short-hairpin-RNA (shRNA) mediated knockout of a specific oncogenic pathway [1, 2]. Moreover, *in vivo* models provide an irreplaceable tool in preclinical testing of therapeutics for which *in vitro* tests are not (completely) adequate, e.g. immunotherapeutics, pro-drugs, drugs acting via the CXCR4/CXCL12 axis, antiangiogenic drugs etc.

Immunodeficient mice currently represent a standard assay for studying the engraftment of primary leukemic cells obtained from patient samples [3, 4]. Although successful engraftment of primary leukemic cells is usually achievable, it has been shown that the murine microenvironment is still not a perfect substitute for the human hematopoietic niche. For example, even the latest mouse models are not capable of correct recapitulation of complex clonality in acute myeloid leukemia (AML) or even of providing satisfactory engraftment success rates and disease recapitulation in the case of chronic lymphocytic leukemia (CLL) [5–7]. Therefore, one of the obvious options for improvement is to provide a functional human microenvironment in the mouse recipients, as has been already demonstrated with the subcutaneous implantation of 3D porous biomaterials covered with human mesenchymal stromal cells (MSC) that create a bone- or bone-marrow-like human tissue supporting the engraftment of hematopoietic and leukemic cells [8, 9]. This concept of bone formation has been widely documented in bone-regenerative medicine. However, research on hybrid mice bearing ectopic bones is still rather uncommon and thus this review details and discusses this new promising research trend.

The discussion in this work focuses on myeloid malignancies and multiple myeloma, as to authors' knowledge other types of hematological malignancies have not been studied using this model of hybrid mouse. Although, for example in the case of CLL with its high bone marrow (BM) microenvironment-dependency, the use of this or similar models is probably only a matter of time.

Applicable biomaterials

The basic purpose of the use of biomaterials in bone-regenerative medicine is to heal severe bone fractures

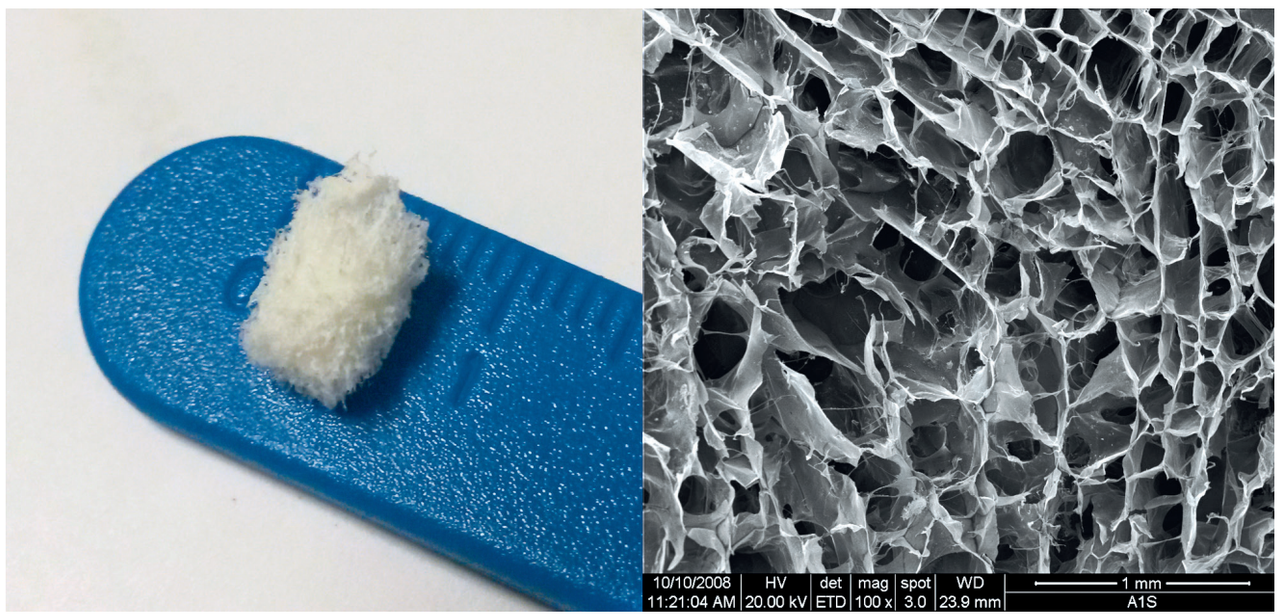


Figure 1 Macroscopic (left) and cross-section microscopic view obtained by scanning electron microscopy (SEM) (right) of a collagen based scaffold material

(SEM photography re-edited according to ref. 12)

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and/or shorten patient recovery time. There is a consensus on the basic parameters of these materials – presence of interconnected macropores, approximately in the range of 200–400 μm and the presence of micropores below 20 μm which have been shown to be essential in multiple works [10–12]. Probably the most straight-forward scaffold material in this case is the natural bone component – calcium phosphate, which is most often used in both its less bioactive form of hydroxyapatite and its better resorbing form of β--tricalcium phosphate (TCP). Further materials also include biopolymers, such as collagen and fibrin and synthetic polymers, for example poly (lactic acid) or polycaprolactone (see Fig. 1) [12, 13]. Combination of materials (for example polycaprolactone and calcium phosphate) can be beneficial and may provide even better mechanical and biological properties of the resulting matrix [14, 15]. Furthermore, incorporation of various soluble growth factors, gradually released from the material, can provide a wide spectrum of study options. For example, bone morphogenetic proteins (BMP) or vascular endothelial growth factor (VEGF) have been shown to induce more profound bone formation and vascularization [16]. The listing of all the applicable materials and their modifications is not the aim of this work, but it should be pointed out that for human bone regeneration, material strength and biodegradability play an important role, whereas in the case of the hybrid-mouse these two parameters are rather irrelevant. Also, the use of MSC in human regenerative medicine represents a significant regulatory obstacle, whereas in the hybrid-mouse model it is not limited and cells may even be subject to further genetic modifications.

Mesenchymal stromal cells

In the case of hybrid mouse model development, MSC are used for their osteogenic potential, although their differentiation capabilities also include chondrogenic and adipogenic lineage [17]. MSC have been found in most tissues, however their extraction is mainly performed from bone marrow or adipose tissue and cord blood. Since no specific surface antigen has been characterized, the basic identification of these cells is defined as:

1. adherence to plastic in tissue cultures,
2. ability to differentiate into the three lineages,
3. and the expression of CD105, CD73, CD90 antigens and simultaneous lack of markers defining other cell populations – CD45, CD34, CD14 or CD11b, CD79alpha or CD19, and HLA-DR [18].

Nevertheless, with increasing knowledge, it is being shown that MSC of diverse origin may also present different phenotypes, not strictly abiding to the formerly proposed definition [17].

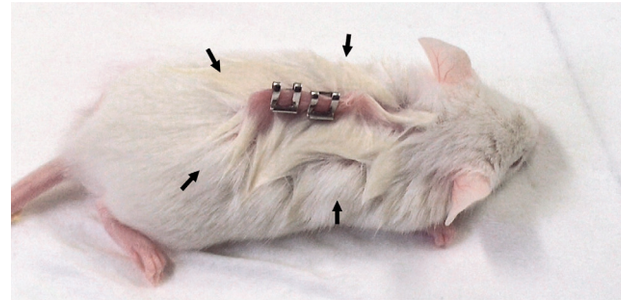


Figure 2 Example of scaffold implantation onto the back of a NSG mouse
The arrows show the approximate subcutaneous location of the matrices.
Figure 1 and Figure 2 attached as .tif files. Copyright agreement for Figure 1 attached as .pdf. file.

Engraftment of hematopoietic or leukemic cells in hybrid mice

In one of the published approaches, formation of humanized microenvironment was based on calcium phosphate matrices in the form of 2–3 mm particles seeded with 0.2 million MSC each [11, 19]. To prime the cells for osteogenic lineage, the seeded scaffolds were cultured for 7 days in osteogenic medium and then implanted on the backs of nude Hsd-cpb:NMRI-nu mice [19]. The creation of new humanized bone-like tissue was subsequently confirmed by histological analysis, where after six weeks the tissue on the implanted constructs formed bone layers with present hematopoietic and fat cells. The model was further evaluated by engraftment of primary multiple myeloma (MM) cells, chosen as an ideal hematologic malignancy for its strong dependence on the BM microenvironment [8]. All 7 MM patient samples were reported to engraft in the ectopic niche and only one also showed parallel engraftment in murine BM. Interestingly, the artificial bone populated by MM cells showed defects and higher occurrence of osteoclasts, which corresponds with the common pathology of this disease. Also, the engrafted disease showed the same response/resistance to therapeutic agents as observed in the patients. The study thus confirmed the intended functionality of the newly created ectopic bone, while reproducing the original disease characteristics. The same model was also assessed using cord blood $CD34^+$ cells transduced with BCR/ABL1 and enabled propagation of leukemia, while in unmodified NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice, a second mutational hit besides the BCR/ABL1 incorporation was required [20]. The same study also

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showed that a primary blast crisis chronic myeloid leukemia (BC CML) sample produced immature blast-like cells in the scaffolds, while more mature cells were present in the murine BM. This nicely demonstrates the superior capacity of the hybrid mice for accurate recapitulation of leukemia.

Another pioneering study published by Vaiselbuh et al. tested the engraftment of primary AML sample/s and was performed with synthetic porous polyurethane scaffolds [9]. Consistently with the previously mentioned works, the MSC seeded matrices showed characteristics of bone marrow tissue at eight weeks and when a leukemia sample was injected into the blood stream, the leukemic cells homed specifically into the scaffolds, while no engraftment was observed in the native mouse tissues when analyzed at 8 weeks. It was only at 4 months that leukemic infiltration was also present in the mouse tissues. Another interesting observation of this study was that large subcutaneous tumors developed after 4 months if the AML cells were injected directly into the implantation sites. The tumors consisted almost exclusively of human CD45 positive cells, and had no other evident adverse effect on the animals. Since the authors presumably used only one AML patient sample, it would be very interesting to show if a wider variety of samples shows selective homing to the humanized niche after i.v. injection as AML is otherwise known to commonly engraft normal non-implanted NOD.CB17-*Prkdc*^{scid} (NS) mouse recipients, as those used in this study. Similarly, it would be interesting to test whether the solid tumor formation seen with this model is a sample or disease specific characteristic and to get more insights on this process. On the cellular level, this study provided an observation, which nicely corresponds to the current understanding of quiescent leukemic stem cells – namely that they are protected by the niche. The authors here found that many of the AML cells maintained tight contact with the MSC and showed negativity for mitosis staining, while other non-adjacent AML cells were positive for mitosis staining. Moreover, a repeated injection of SDF-1α into the implantation sites showed a positive effect and dramatically increased MSC proliferation, forming thick layers with more adjacent AML cells. On the contrary, plerixafor (CXCR4 antagonist) treated scaffolds were covered only with a thin and disrupted stromal lining. Control untreated scaffolds also showed a thin but compact MSC layer, however with fewer leukemic cells than the SDF-1α treated ones. This finding may indicate that SDF-1α may actually be used as an agent to support or even accelerate tissue formation on the scaffolds and may deserve further attention.

A very unique scaffold design was applied by Nichols et al. who used fabricated polyacrylamide hydrogel scaffolds coated with collagen and designed to maintain a defined and uniform spatial geometry [21]. In the initial *in vivo* study, the authors interestingly used an approach where they implanted the scaffolds with feeder cell lines already seeded with CD34$^+$ cells. However, this method was abandoned in the following work, where classic seeding with MSC was adopted and HSCs were injected only after the development of the bone-like tissue *in vivo* on the matrices, perhaps indicating worse performance of the former method [22]. Nevertheless, in both cases, the scaffolds were shown to develop vascularity and supported engraftment of HSCs as well as of an erythroleukemic cell line TF-1a. Importantly, the work demonstrated a challenging, but very beneficial technical solution whereby the processes inside the live scaffolds were observed by intravital microscopy, showing how the leukemic cells adhered to the vasculature after their injection.

A further unorthodox approach to the creation of human bone-like tissue in mice was presented in another study. It in fact omitted the use of a solid scaffold and instead a subcutaneous injection of mesenchymal and endothelial cells mixed with matrigel, a gelling protein mixture, was used, without prior osteogenic priming [23]. The formation of bone in the NSG mice was then observed with microcomputed tomography, further enhanced by *in vivo* staining with a fluorescent hydroxyapatite targeting agent. After 8 weeks, a bone-like tissue with trabecular structure was present at the injection site. Contrary to previous studies, engraftment of human CD45 positive cells derived from injected healthy BM mononuclear cells (MNC) could be seen in both the mouse femurs and the ectopic human niches at the same time, at 4 and 11 weeks. The ectopic bones were further tested by injection of the Luc-GFP transfected AML cell line MOLM13, which was injected 6 weeks after introduction of the bone forming mixture. After a 2 week incubation period, infiltration could be detected in the spleen and liver with ample infiltration and severe hypoxia detected in the femurs and also the extramedullary bones. The role of hypoxia was further elegantly assessed by implanting MSC with the shRNA-silenced HIF-1α gene, a hypoxia response mediator, into one flank of the mice and control MSC into the opposite flank of the same mouse. This resulted in 50% less engraftment in the modified ectopic niche and was suggested to be a result of an observed 30% lower expression of SDF-1α measured in the modified MSC.

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CONCLUSIONS

The implementation of a humanized niche into mice recipients appears to be a new promising approach for obtaining a model capable of faithfully simulating a patient's leukemia. The reviewed works showed an almost uniform approach – using MSC based extramedullary bone creation. However, since hematopoiesis is active not only in bone marrow but also in the liver and spleen, the question remains whether specific modeling of bone or bone-marrow environment is in fact crucial, or whether there is a simpler general stromal structure that would be perfectly sufficient for this purpose. An answer to this question will only become available with a more profound understanding of the hematopoietic niche, its composition and functioning. However, this is not available at this time.

When considering the described engraftment of leukemic cells, the primary MM and AML samples showed specific homing to the introduced niches, as described in the first two works. Of the MM samples, only one aggressive relapse/refractory sample engrafted also in the murine BM. Engraftment of the primary AML sample in the other study was present in mouse BM only after a long time (in contrast to the ectopic niches). It should be noted that these two studies employed non-irradiated mice, while in the other studies where engraftment was shown in both ectopic niches and mouse BM the mice had been irradiated before the studied cells were injected. It is therefore questionable, whether the non-irradiated hybrid mouse can distinguish the most aggressive samples in this manner.

Based on the summarized data, the most important features of the presented hybrid-mouse model are that it allows a more faithful simulation of patient leukemia than the current immunodeficient mouse strains and that it provides the possibility of targeted modification and study of the hematopoietic niche. It will therefore be interesting to see what further discoveries this model brings; although its truly widespread use is not expected given its level of technical complexity.

REFERENCES

1. Zuber J, Rappaport AR, Luo W, et al. An integrated approach to dissecting oncogene addiction implicates a myb-coordinated self-renewal program as essential for leukemia maintenance. Genes Dev 2011; 25: 1628–1640.
2. Li L, Wang L, Li L, et al. Activation of p53 by sirt1 inhibition enhances elimination of cml leukemia stem cells in combination with imatinib. Cancer Cell 2012; 21: 266–281.
3. Sarry JE, Murphy K, Perry R, et al. Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in nod/scid/il2rgammac-deficient mice. J Clin Invest 2011; 121: 384–395.
4. Chu S, McDonald T, Lin A, et al. Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment. Blood 2011; 118: 5565–5572.
5. Woiterski J, Ebinger M, Witte KE, et al. Engraftment of low numbers of pediatric acute lymphoid and myeloid leukemias into nod/scid/il2rcgammanull mice reflects individual leukemogenecity and highly correlates with clinical outcome. Int J Cancer 2013; 133: 1547–1556.
6. Klco JM, Spencer DH, Miller CA, et al. Functional heterogeneity of genetically defined subclones in acute myeloid leukemia. Cancer Cell 2014; 25: 379–392.
7. Bertilaccio MT, Scielzo C, Simonetti G, et al. Xenograft models of chronic lymphocytic leukemia: Problems, pitfalls and future directions. Leukemia 2013; 27: 534–540.
8. Groen RW, Noort WA, Raymakers RA, et al. Reconstructing the human hematopoietic niche in immunodeficient mice: Opportunities for studying primary multiple myeloma. Blood 2012; 120: e9–e16.
9. Vaiselbuh SR, Edelman M, Lipton JM, Liu JM. Ectopic human mesenchymal stem cell-coated scaffolds in nod/scid mice: An in vivo model of the leukemia niche. Tissue Eng Part C Methods 2010; 16: 1523–1531.
10. Woodard JR, Hilldore AJ, Lan SK, et al. The mechanical properties and osteoconductivity of hydroxyapatite bone scaffolds with multi--scale porosity. Biomaterials 2007; 28: 45–54.
11. Yuan H, Van Den Doel M, Li S, et al. A comparison of the osteoinductive potential of two calcium phosphate ceramics implanted intramuscularly in goats. J Mater Sci Mater Med 2002; 13: 1271–1275.
12. Prosecka E, Rampichova M, Vojtova L, et al. Optimized conditions for mesenchymal stem cells to differentiate into osteoblasts on a collagen/hydroxyapatite matrix. J Biomed Mater Res A 2011; 99: 307–315.
13. Lee SH, Shin H. Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. Adv Drug Deliv Rev 2007; 59: 339–359.
14. Xue W, Bandyopadhyay A, Bose S. Polycaprolactone coated porous tricalcium phosphate scaffolds for controlled release of protein for tissue engineering. J Biomed Mater Res B Appl Biomater 2009; 91: 831–838.
15. Roohani-Esfahani SI, Nouri-Khorasani S, Lu Z, Appleyard R, Zreiqat H. The influence hydroxyapatite nanoparticle shape and size on the properties of biphasic calcium phosphate scaffolds coated with hydroxyapatite-pcl composites. Biomaterials 2010; 31: 5498–5509.
16. Kempen DH, Lu L, Heijink A, et al. Effect of local sequential vegf and bmp-2 delivery on ectopic and orthotopic bone regeneration. Biomaterials 2009; 30: 2816–2825.
17. Keating A. Mesenchymal stromal cells: New directions. Cell Stem Cell 2012; 10: 709–716.
18. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. Cytotherapy 2006; 8: 315–317.
19. Prins HJ, Rozemuller H, Vonk-Griffioen S, et al. Bone-forming capa-

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city of mesenchymal stromal cells when cultured in the presence of human platelet lysate as substitute for fetal bovine serum. Tissue Eng Part A 2009; 15: 3741–3751.
20. Groen RWJ, Jaques J, Yuan H, et al. Mouse versus human extrinsic cues dictate transformation potential in bcr-abl/bmi1-induced leukemia in humanized xenograft models. Blood 2013; 122: 515.
21. Nichols JE, Cortiella J, Lee J, et al. In vitro analog of human bone marrow from 3d scaffolds with biomimetic inverted colloidal crystal geometry. Biomaterials 2009; 30: 1071–1079.
22. Lee J, Li M, Milwid J, et al. Implantable microenvironments to attract hematopoietic stem/cancer cells. Proc Natl Acad Sci USA 2012; 109: 19638–19643.
23. Chen Y, Jacamo R, Shi YX, et al. Human extramedullary bone marrow in mice: A novel in vivo model of genetically controlled hematopoietic microenvironment. Blood 2012; 119: 4971–4980.

Authors' contribution

M. Č. – *manuscript preparation, other authors* (D. D., L. S., Z. Š., J. B., M. P., J. M., Z. R.) – *revisions and proof-checking.*

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