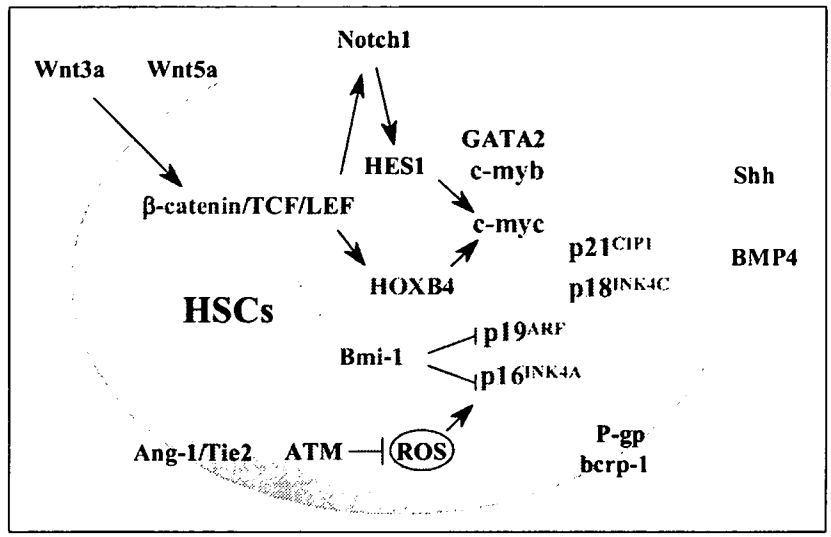

Figure 2. Regulation of Stemness by Intrinsic Factors in HSCs.

This decoy peptide was delivered into about 70% of $CD34^+$ cells. By examining the expression of HOX target genes c-*myc* and *p21*$^{waf1/cip1}$, we confirmed that decoy peptide enhanced HOX functions. After 7-day culture in serum-free medium containing a cytokine cocktail, the decoy peptide increased numbers of $CD34^+$ cells and primitive multipotent progenitor cells (CFU-Mix) approximately two-fold compared to control cultures. Furthermore, $CD34^+$ cells treated with the decoy peptide reconstituted hematopoiesis in NOD/SCID mice more rapidly and more effectively than control cells (more than two-fold greater efficiency as determined by a limiting dilution method). In addition, decoy peptide-treated $CD34^+$ cells were able to repopulate secondary recipients. Therefore, the decoy peptide will be a promising novel tool for the safe *ex vivo* expansion of human HSC/HPCs in combination with growth factors and/or other approaches.

In addition to HOXB4, other HOX homeobox transcription factors play important roles in the proliferation and differentiation of hematopoietic cells [102,103], however, their physiological functions and roles in leukemogenesis have not been elucidated. Bmi-1, a member of the Polycomb Group family of transcriptional repressors [104], was recently shown to be essential for maintenance of adult self-renewing HSCs [105]. Although the number of HSCs in the fetal liver of Bmi-$1^{-/-}$ mice was normal, the number of HSCs was markedly reduced in postnatal Bmi-$1^{-/-}$ mice. Furthermore, transplanted fetal liver and bone marrow cells obtained from Bmi-$1^{-/-}$ mice were able to contribute to hematopoiesis only transiently. Regarding this mechanism, in accord with the previous data obtained from embryonic fibroblasts [106], the microarray analysis on the BM mononuclear cells showed that the expression of $p16^{INK4A}$ (p16) and $p19^{ARF}$ was upregulated in Bmi-$1^{-/-}$ BM cells. On the other hand, it was reported that the increased expression of Bmi-1 promotes HSC self-renewal in mouse studies [107]. Furthermore, very recently, Rizo, et al. reported that overexpression of Bmi-1 induced *ex vivo* expansion for over 4 month of human CB $CD34^+$ cells in liquid cultures without loosing the potential of stem/progenitor cells [108]. Their data indicate that Bmi-1 is one of the important modulator of human HSCs self-renewal and suggest that it can be a potential target for therapeutic manipulation of human HSCs.

Roles of Cell Cycle Inhibitors, p21 and p27, in HSCs and Progenitor Cells

During the last decade, a number of cell cycle regulatory molecules such as cyclins, cyclin-dependent kinases (CDKs) and CKIs have been identified and their roles and regulation have been well characterized in various types of cells [109-111].

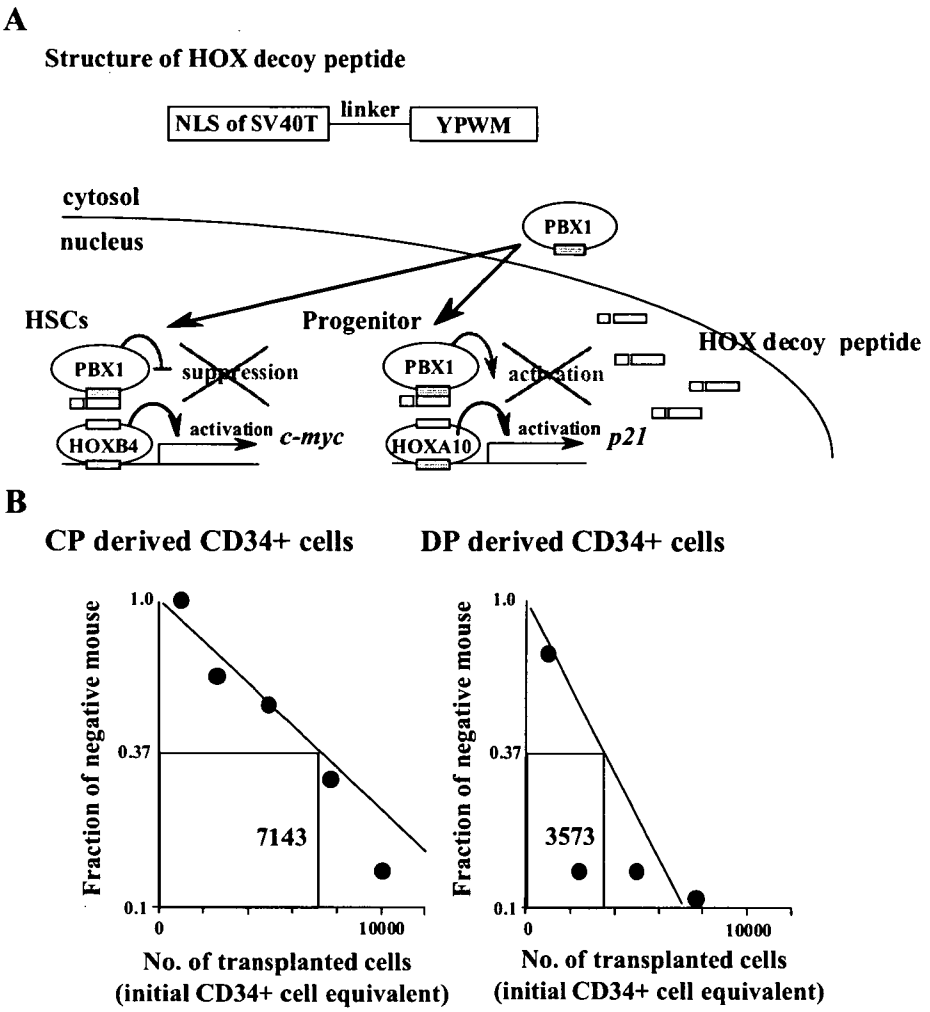

Figure 3. Peptide minetics and *ex vivo* expansion of human HSC/HPCs. A. The HOX decoy peptide contains the YPWM motif of HOX, utilized for the cooperative interaction with PBX1, and the nuclear localization signal (NLS) of the SV40 large T antigen. PBX1 negatively regulates HOXB4-mediated c-*myc* transcription in HSCs, while it promotes HOXA10-mediated $p21^{waf1/cip1}$ expression in myelomonocytic progenitors. Hox decoy peptide was supposed to inhibit both positive and negative effects of PBX1 on HOX-mediated transcription. B. The frequency of LTR-HSCs was calculated to be 1/7143 control peptide (CP)-treated CB $CD34^+$ cells 6 weeks after the transplantation to NOD/SCID mice. In contrast, the frequency of LTR-HSCs in HOX decoy peptide (DP)-treated cells was calculated to be 1/3573. Accordingly, the expansion of LTR-HSCs by the decoy peptide was estimated as 2.0-fold.

Cell cycle is positively regulated by CDKs associated with cyclins, and their activities are negatively regulated by CKIs also included in these complexes at the same time. CKIs are classified into two families based on their structures and CDK targets. One class of inhibitors including p21, p27, and $p57^{KIP2}$ share a CDK2-binding motif in the N-terminus and inhibit the activities of cyclinD-, E-, and A-dependent kinases. The other class of inhibitors also known as the INK4 family, including p16, p15, $p18^{INK4C}$ (p18), and $p19^{INK4D}$, contain fourfold ankyrin repeats and specifically inhibit CDK4 and CDK6. Members of both families are important for executing cell cycle arrest in response to a variety of stimuli such as DNA damage, contact inhibition, and TGF-β1treatment. Embryonic fibroblasts obtained from $p21^{-/-}$ mice had a defect in their ability to achieve cell cycle arrest after irradiation [112,113], and antisense oligonucleotides against p21 was shown to release human mesenchymal cells from G0 phase [114]. As for the roles for p21 in hematopoiesis, the

expression level of p21 was initially reported to be low in $CD34^+$ cells [115,116], and $p21^{-/-}$ mice did not exhibit an apparent hematologic defect [112,113]. However, in a subsequent analysis, Cheng *et al* found that p21 was highly expressed in the quiescent stem cell-like fraction of BM cells [117]. They also found that, under normal homeostatic conditions, the proportion of quiescent HSCs in G0 phase was reduced and that total number of HSCs increased in $p21^{-/-}$ mice. In accord with these findings, when $p21^{-/-}$ mice were treated with 5-FU, the survival percentage was much lower in $p21^{-/-}$ mice than in littermate controls. They also directly assessed self-renewal ability of HSCs using a serial transplantation approach. As a result, no mice transplanted with $p21^{-/-}$ BM cells survived after the fifth transplant due to the exhaustion of HSC population, whereas those transplanted with $p21^{+/+}$ BM cells had a 50% survival. Together, these results indicate that p21 is a key molecule that restricts cell cycle entry of HSCs, thereby keeping their pool and preventing their exhaustion under certain stress conditions. In consistent with these results, Stier *et al.* reported that p21-antisense transduced by the lentivirus vector released human $CD34^+CD38^-$ cells from the quiescent state and induced an approximately 2~3-fold expansion of SRCs without loosing multipotency [118], However, further research is required to determine whether human HSCs lacking p21 have the long-term reconstitution abilities.

p27 is molecularly distinct from p21 in its carboxyl terminus; it interacts with similar, though not identical, cyclin-CDK complex and lacks p53-regulated expression. In hematopoietic system, the expression of p27 is observed in more mature progenitors than that of p21 [115,116]. Mice homozygously lacking p27 have a larger body and hyperplasia of most organs including hematopoietic organs [119-121]. In striking contrast to $p21^{-/-}$ mice, the number, cell cycling and self-renewal of HSCs were normal in $p27^{-/-}$ mice, while these mice had an increase in hematopoietic progenitor cells [122]. In addition, these progenitor cells in $p27^{-/-}$ mice were more proliferative than $p27^{+/+}$ progenitor cells. Furthermore, progenitor cells from $p27^{-/-}$ mice were able to expand and regenerate hematopoiesis after serial transplantation, while $p27^{+/+}$ progenitors were markedly depleted. Thus, p21 and p27 govern stem and progenitor cell populations divergently.

Roles for the INK4 Family in Self-Renewal of HSCs and as Tumor Suppressor Genes

In addition to p21 and p27, the INK4 family of CKIs is also implicated in the regulation of HSCs numbers and self-renewal. Mice deficient for p18 had an increased number of HSCs in the bone marrow. Also, competitive repopulation assays showed that $p18^{-/-}$ HSCs were far more competitive than normal HSCs with 14-fold activities. In contrast to $p21^{-/-}$ HSCs, the exhaustion of $p18^{-/-}$ HSCs was not observed during serial bone marrow transplants, indicating that p18 is a strong inhibitor limiting the potential of stem cell self-renewal *in vivo* [123]. From this result, it was speculated that downregulation of the expression of p18 in HSCs, using antisense oligonucleotide or small interfering RNA (siRNA), would be useful for the *ex vivo* expansion of HSCs.

p16 is highly expressed in $CD34^+$ cells, and its expression is downregulated during differentiation process towards all lineages [124]. Nontheless, since $p16^{-/-}$ mice did not show an apparent abnormality in hematopoiesis, p16 was supposed to be dispensable for the quiescence of HSCs [125,126]. However, Ito et al. found that p16 was essential for reconstitution activities of HSCs but not for proliferation or differentiation of progenitors from the analysis of mice lacking one of the cell cycle check point kinase, "ataxia telangiectasia mutated" (Atm) [127]. In this analysis, $Atm^{-/-}$ mice older than 24 weeks developed progressive bone marrow failure due to a defect of HSC function, which was associated with elevated reactive oxygen species (ROS). Furthermore, they proved p16-retinoblastoma (Rb) pathway activated by ROS was critical for the defective function of HSCs. From these results, they concluded that the self-renewal ability of HSCs depend on ATM-mediated inhibition of oxidative stress and p16-RB pathway. Furthermore, Janzen et al. recently demonstrated that ageing causes an increase in p16 and intrinsic p16 suppress the proliferation of HSC/HPCs in the bone marrow. And they supposed that inhibition of p16 may ameliorate the physiological impact of

ageing on stem cells and thereby improve injury repair in aged tissue [128].

In contrast to the expression pattern of p16, the expression of p15 is not detected in $CD34^+$ cells, but increased specfically during myeloid differentiation [124,129]. However, the functional role of p15 in HSCs remained to be clarified. Both p16 and p15 inhibit the function of cyclin D-CDK4/6 complex and suppress the phosphorylation of pRb, thereby inducing cell cycle arrest at G0/G1 phase. Especially, under tumorgenetic stress such as the presence of oncogenic ras gene, p16 and p15 are induced to express and suppress tumor progression through the induction of premature senescence [130,131]. With these activities, both p16 and p15 are supposed to act as tumor suppressor genes. In fact, inactivation and/or deletion of p16 and p15 genes are observed in various human cancers very frequently [132,133]. As for hematological malignancies, their defects caused by the homozygous deletion or methylation were observed in a substantial proportion of AML, ALL, and myelodysplastic syndromes (MDS) cases [134-136]. These results indicate that appropriate cell cycle control, particularly at the stage of stem/progenitor cells, is required for maintaining normal hematopoiesis, and have to be uppermost when manipulating HSCs *ex vivo*.

Pilot Trials of Transplantation with *Ex Vivo* Expanded CB Cells

To date, three groups of investigators have utilized *ex vivo*-amplified CB HSCs for transplantation (table 1). Shpall *et al.* isolated $CD34^+$ cells from CB. Then, forty percent of the isolated cells were expanded in medium containing SCF, G-CSF, and TPO for 10 days, and the remaining 60% were immediately transplanted or stored frozen until transplantation [137]. After high-dose chemotherapy, 37 patients (25 adults, 12 children) were transplanted with expanded $CD34^+$ cells and non-expanded cells with a median dose of 0.99×10^7 nucleated cells per kilogram. The median time to engraftment of neutrophils (neutrophil count >500/μl) was 28 days (range 15-49 days) and that of platelets (platelet >20,000/μl) was 106 days (range 38-345 days). From this study, the authors concluded that, although the transplantation with *ex vivo* expanded CB cells was feasible and safe, expanded HSCs did not improve the time to engraftment in recipients. In a phase I trial, Jaroscak *et al.* transplanted CB HSCs expanded by PIXY321, FL, and EPO into 28 patients with a median dose of 2.4×10^7 nucleated cells per kilogram [138]. They also concluded that the *ex vivo*-expanded CB HSCs were not effective in shortening the recovery period, probably due to insufficient expansion of $CD34^+$ cells in their culture system. In contrast, Shpall *et al.* expanded $CD133^+$ cells isolated from CB in liquid culture containing with SCF, IL-6, FL, and polyamine copper chelator, TEPA for 21 days, which led to the expansion of a cell population that displays phenotypic and functional characteristics of HSC/HPCs [139]. As a result of transplantation into the 10 patients, the median time to engraftment was 27 days for neutrophils (range 16-46 days) and 48 days for platelets (range 27-96 days). This preliminary result showed that a shorter time to neutrophil engraftment was correlated with total TNC per kilogram infused, and a trend with $CD34^+$ cells per kilogram infused. These pilot studies indicated that a novel strategy, which can expand HSCs more efficiently without losing their functions and properties, is absolutely prerequisite for the useful clinical application of HSC/HPCs expansion.

Conclusion

Owing to the recent advance in stem cell biology, various molecules involved in self-renewal of HSCs have been identified. Also, it has been clarified how the activities of these molecules are regulated. So, the establishment of a novel useful strategy for *ex vivo* expansion of HSCs and its clinical application will be realized in the near future (Figure 4), However, further studies are required to disclose the whole feature of stemness regulation in HSCs. These studies would undoubtfully enable us to utilize HSCs more efficiently.

Table 1. Pilot trials of UCB transplantation with *ex vivo* expanded CB cells

Group	Shpall et al. [136]	Jaroscak et al. [137]	Shpall et al. [138]
Cytokine cocktail	SCF+G-CSF +TPO	GM-CSF+IL-3 +FL+EPO	SCF+IL-6+FL +TPO+TEP A
Culture duration	10 days	12 days	21 days
Fold expansion ofTNCs*	56 (0.1-278)	2.05 (0.06-10.19)	207 (2-616)
Fold expansion of CD34*	4 (0.1-20)	0.5 (0.09-2.45)	N.A.
Infused TNCs(x10 ^{7}/kg)*	0.99 (0.28-8.5)	2.4 (1.0-8.5)	1.8 (1.1-6.1)
Infused CD34(x10 ^{5}/kg)*	1.04 (0.97-31.1)	0.22 (0.001-2.59)	1.6 (0.4-49.9)
No. of enrolled patients	37	2 8	10
Engraftment			
Neutro>500*	Day 28 (15-49)	Day 22 (13-40)	Day 27 (16-46)
Plt>50,000*	Day106 (28-345)	Day 94 (41-370)	Day 48 (27-96)
Graft failure	0/30	3/24	0/7
acute GVHD (>II)	40%	36%	43%

* median (range).
TNCs: total nuclear cells.
N.A.: no assessment.

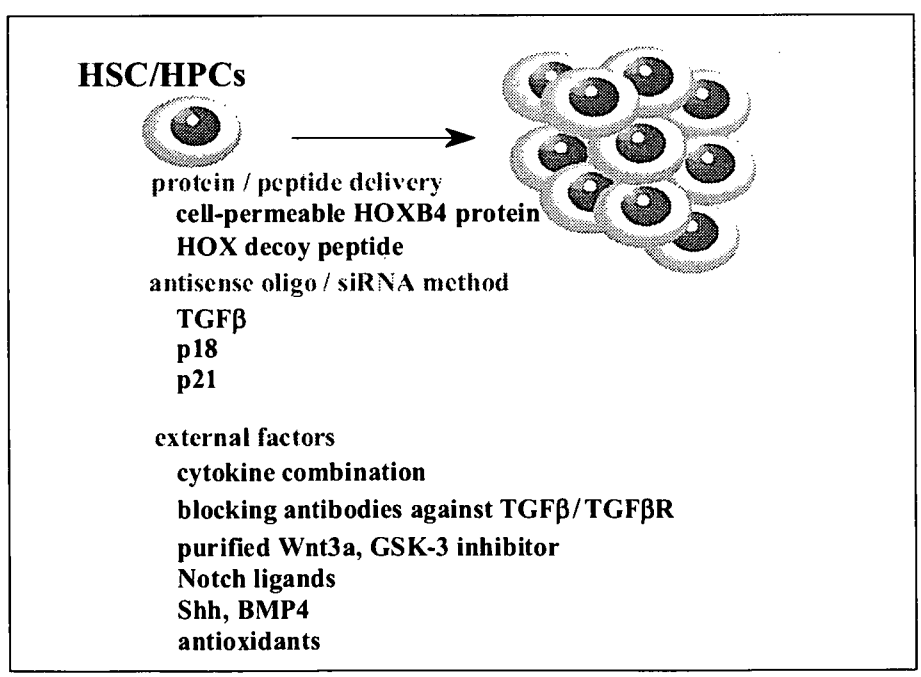

Figure 4. Strategies of therapeutic HSC/HPCs expansion.

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2. 基 礎 編

造血幹細胞の体外増幅

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2 基礎編

造血幹細胞の体外増幅*

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Key Words : cord blood, hematopoietic stem/progenitor cells, *ex vivo* expansion, translational research

はじめに

幹細胞は自己複製能と多分化能とを併せもつ細胞と定義され，これまでにさまざまな組織においてその存在が明らかにされている．さらに造血幹細胞においては，その生体内での増殖や分化が，細胞外の液性因子，造血幹細胞を取り巻く支持細胞や組織との相互作用，および細胞内の転写因子やシグナル伝達分子などにより厳密に制御されていることが明らかにされつつある．近年，造血幹細胞のもつ自己複製能を利用して*ex vivo*で造血幹/前駆細胞を増幅し，移植医療に応用しようとする研究が盛んに行われ，海外では臨床研究も進められている．造血幹細胞の*ex vivo*増幅は，新たな移植細胞ソースとしての利用だけでなく，造血幹細胞の遺伝子治療，さらにはその可塑性を利用した再生医療など多方面への応用が期待されている技術である．本稿では，造血幹細胞の*ex vivo*増幅の現状，およびわれわれが先端医療センターにおいて取り組んでいる*ex vivo*増幅臍帯血を用いた臨床研究について概説する．

造血幹細胞の体外増幅研究の現状

造血幹細胞の体外増幅方法は，培地へのサイトカインなどの液性因子の添加や支持細胞との共培養などによる外的因子操作法，および遺伝子やタンパク導入などによる内的因子操作法に大きく分類される[1)~3)]．

1．外的因子操作による造血幹細胞の増幅

(1)液性因子を用いた体外増幅法

これまでさまざまなサイトカインの組み合わせにより，臍帯血から分離したCD34陽性造血幹/前駆細胞の増幅効果が検討された．現在，ヒト造血幹細胞が有する未分化能を評価できる系としてもっとも優れているのが，NOD/SCIDマウスへの異種間移植系であり，マウス体内においてヒト造血を再構築可能な細胞数〔SCID repopulating cells；SRC〕を算出することで，移植した細胞集団にどれだけの造血幹細胞が含まれていたかを測定することが可能である．Uedaらは，臍帯血由来のCD34陽性細胞をSCF, TPO, Flk2/Flt-3 ligand(FL)，IL-6/sIL-6R存在下で1週間培養することにより，SRCを4倍以上に増幅できることを報告し[4)]，また移植後6か月以上経過してもマウス末梢血中にある一定の割合でヒト細胞が認められたことから，この系を用いることにより比較的長期の骨髄再構築能を維持した造血幹細胞が増幅可能であると考えられている[5)]．

* *Ex vivo* expansion of human umbilical cord blood hematopoietic stem/progenitor cells.

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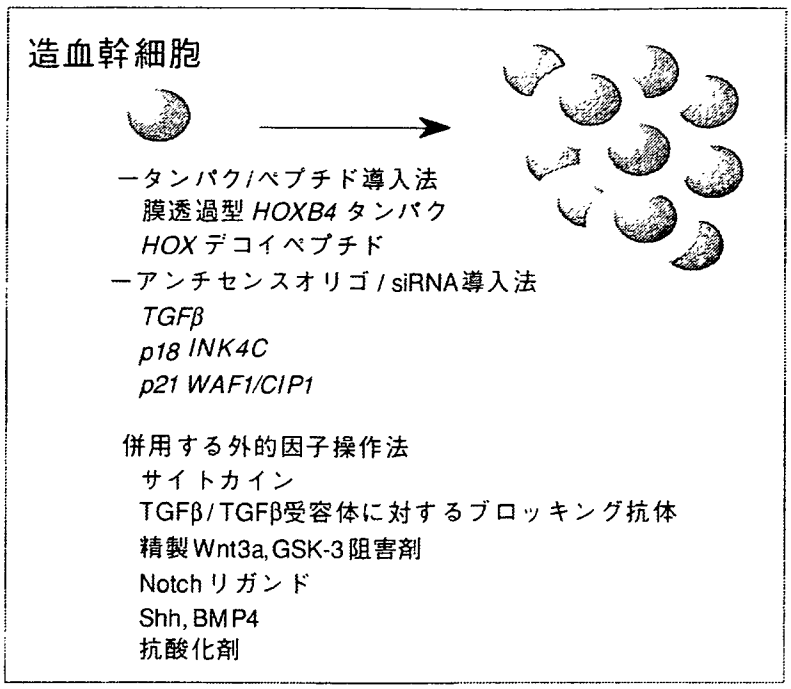

図1 臨床応用に向けた造血幹細胞の体外増幅法

(2)支持細胞との共培養による体外増幅法

造血幹細胞は骨髄中のnicheと呼ばれる特殊な場所に存在し，幹細胞としての性質を維持していると考えられている．近年nicheを構成する細胞群や，nicheでの造血幹細胞の未分化性維持に関与する分子が明らかにされつつあり[6]，支持細胞との共培養や分子の固相化などの加工培養容器を用いることによる効率的な造血幹/前駆細胞の体外増幅法の開発が期待されている[7]．さらに，増幅のみならず生着促進効果や長期骨髄再建能の維持を考慮した場合，造血幹細胞を生体内に近い状態で培養することが望ましいと考えられるが，これまで報告されてきたようなMS-5やOP9など異種支持細胞との共培養系は，安全性の面からその応用は制限されてしまう．最近，採取した臍帯血と同じ臍帯より得た臍静脈上皮細胞，human umbilical vein endothelial cell(HUVEC)とを共培養することにより，サイトカインのみで培養した場合よりも効率的に造血幹/前駆細胞の増幅が可能であることが報告されており[8]，今後SRCの増幅効果や生着に及ぼす効果などの検討が期待される．

2．内的因子操作による造血幹細胞の増幅

これまでの実験的な検討や後述する増幅臍帯血を用いた臨床研究の結果から，外的因子操作単独による増幅効率には限界があり，より効率よく造血幹細胞を増幅するためには内的な因子の操作が必要であると考えられている．これまでの内的因子操作は，造血幹細胞の自己複製への関与が明らかにされている遺伝子群をウイルスベクターに搭載し導入する方法を中心に行われてきたが，近年臨床応用に向けた新たな方法の開発も進められている(図1)．

(1)遺伝子導入による体外増幅

これまでに*HOX*転写因子群，*β-catenin*や*Bmi-1*など造血幹細胞の自己複製を促進する因子を強制発現する，あるいは$p21^{CIP1/WAF1}$や$p18^{INK4C}$など造血幹細胞の静止期維持に機能する因子の発現をアンチセンスオリゴなどの導入により抑制することで，マウスおよびヒト造血幹細胞の増幅が可能であることが報告されている．本稿では，紙面の都合上，*HOXB4*および*β-catenin*について概説する．

造血細胞の増殖，分化に重要なホメオボックス型転写因子*HOX*転写因子群の中で，とくに注目されているのが*HOXB4*である．Antonchukらはマウス骨髄細胞にレトロウイルスベクターを用いて*HOXB4*を過剰発現させ，サイトカイン存在下で14日間培養し，CRU(competitive repopulating units)を測定することにより，造血幹細胞

を培養前の約40倍に増幅することが可能であることを報告した[9]．さらに*HOXB4*を過剰発現させた細胞を移植した場合，白血病の発症をきたすことなく，造血における正常な制御機構を維持していることが明らかとなった．この点が*HOXA9*や*HOXA10*を強制発現させた場合と決定的に異なる点であり，*HOXB4*がとくに注目されている理由の一つである．その後ヒトCD34陽性細胞においても，SRCを約30倍に増幅可能であることが報告されている[10]．

*Wnt/β-catenin*経路は，発生の段階での体軸決定，臓器形成に重要なシグナル経路であることが知られているが，近年生体内における造血幹細胞の発生，維持に関与していることが明らかになってきている．Reyaらはレトロウイルスベクターを用いて恒常的活性型の*β-catenin*を*Bcl-2*トランスジェニックマウスの造血幹細胞に遺伝子導入し，サイトカイン存在下で培養した．限界希釈法により造血幹細胞の増幅率を算出した結果，7日間で8～80倍の増幅が可能であるとしている[11]．一方，恒常的活性型*β-catenin*の導入は，静止期にある造血幹細胞を強制的に細胞周期へと誘導し，さらには成熟血球への分化障害をもたらすことにより，生体における造血幹細胞の枯渇，造血障害をきたすことが報告されており[12][13]，今後*Wnt/β-catenin*経路の活性化による増幅方法を臨床応用するためには，活性化させる期間など厳密な調節が必要であると考えられている．

(2)細胞透過型タンパク導入による体外増幅

ウイルスベクターを用いた遺伝子導入方法は，安全性の問題から現時点での臨床応用は難しいと考えられる．この点を回避するため，細胞透過型タンパクを導入する方法が試みられている．Krosらは可溶性*HOXB4*タンパク(TAT-*HOXB4*)をマウス骨髄細胞培養液に加えることで，細胞内の*HOXB4*タンパクレベルを上昇させ，CRU活性を約4倍上昇させうることを報告しており[14]，今後このようなタンパク導入による内的因子操作と種々外的因子操作を併用した形での増幅方法が，有力かつ実用的な方法になると考えられる．ただし，細胞透過型タンパクの細胞内半減期は非常に短く，持続的に発現させるためには数時間ごとに添加する必要があるなどの課題も残されている．

(3)合成ペプチド導入による体外増幅

われわれは新たな内的因子操作法として，合成ペプチドによる体外増幅法の開発に取り組んでいる．上述した*HOX*転写因子群は，同じホメオボックス型転写因子*PBX1*とヘテロ複合体を形成し，種々の遺伝子発現を正または負に制御していることが知られている．そこでわれわれは，*PBX1*との結合領域部分の*HOX*タンパクのdecoyペプチドを設計，合成した(図2)．ヒト臍帯血CD34陽性細胞に導入し検討した結果，このdecoyペプチドは，導入した細胞の核内で*PBX1*と直接結合し，その*HOXB4*に対する抑制作用を解除すること，さらに比較的長時間にわたり内因性の*HOXB4*の活性を上昇させ，サイトカインのみで増幅した場合と比較して約2倍SRCを増幅させうることが明らかとなった[15]．本法はタンパク導入法と同様に安全であり，かつ簡便な方法と考えられ，現在増幅効率の改善に向けた新たな合成ペプチドの設計，ならびに評価を行っている．

造血幹細胞増幅の臨床的意義

臍帯血移植の短所として移植後の造血回復，とくに血小板の生着が他の造血幹細胞移植と比べ顕著に遅延することが知られている．これまでの報告から，移植細胞数とくにCD34陽性細胞数が多いほど造血回復能が高く，予後も良好と考えられており[16]～[18]，*ex vivo*増幅したCD34陽性造血幹/前駆細胞が移植可能となれば，従来臍帯血移植の対象とならなかった体重の重い患者も対象となるばかりでなく，生着不全の減少や生着日数の短縮が期待される．

同様の考え方で，十分な細胞数を確保することを目的として，複数の臍帯血を同時に移植する試みが行われている(本誌第3章「臍帯血移植」の項を参照)．

*Ex vivo*増幅造血幹/前駆細胞を用いた移植成績(表1)

*Ex vivo*増幅した臍帯血を用いた臨床試験は，まず1997年にShpall[19]，Jaroscak[20]ら2つのグルー

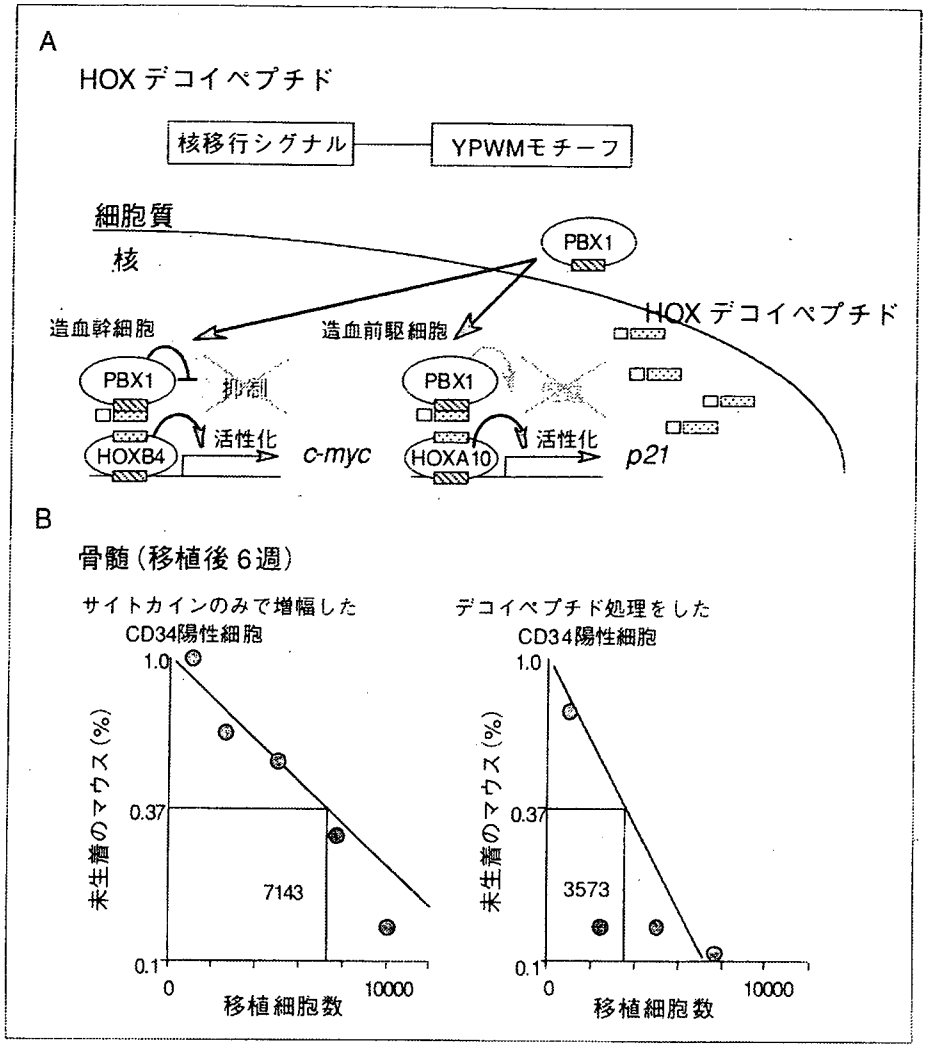

図2　合成ペプチドを用いた内的因子操作法
A：HOXデコイペプチドの構造と予想される作用．B：限界希釈法によるSRCの評価．
（文献[15]より引用）

表1　*Ex vivo*増幅臍帯血を用いた臍帯血移植における培養結果と臨床成績

Group	Shpall[19]	Jaroscak[20]	Shpall[21]
サイトカイン	SCF＋G-CSF＋TPO	GM-CSF＋IL-3＋FL＋EPO	SCF＋IL-6＋FL＋TPO＋TEPA
培養日数	10日間	12日間	21日間
総細胞増幅率（倍）	56（0.1～278）	2.05（0.06～10.19）	207（2～616）
CD34陽性細胞増幅率（倍）	4（0.1～20）	0.5（0.09～2.45）	－
移植総細胞数（$\times 10^7$/kg）	0.99（0.28～8.5）	2.4（1.0～8.5）	1.8（1.1～6.1）
移植CD34陽性細胞数（$\times 10^5$/kg）	1.04（0.97～31.1）	0.22（0.001～2.59）	1.6（0.4～49.9）
移植患者数	37	28	10
生着			
好中球＞500/μl	28日（15～49）	22日（13～40）	27日（16～46）
血小板＞50,000/μl	106日（28～345）	94日（41～370）	48日（27～96）
生着不全	0/30	3/24	0/7
急性GVHD（II度以上）	40%	36%	43%

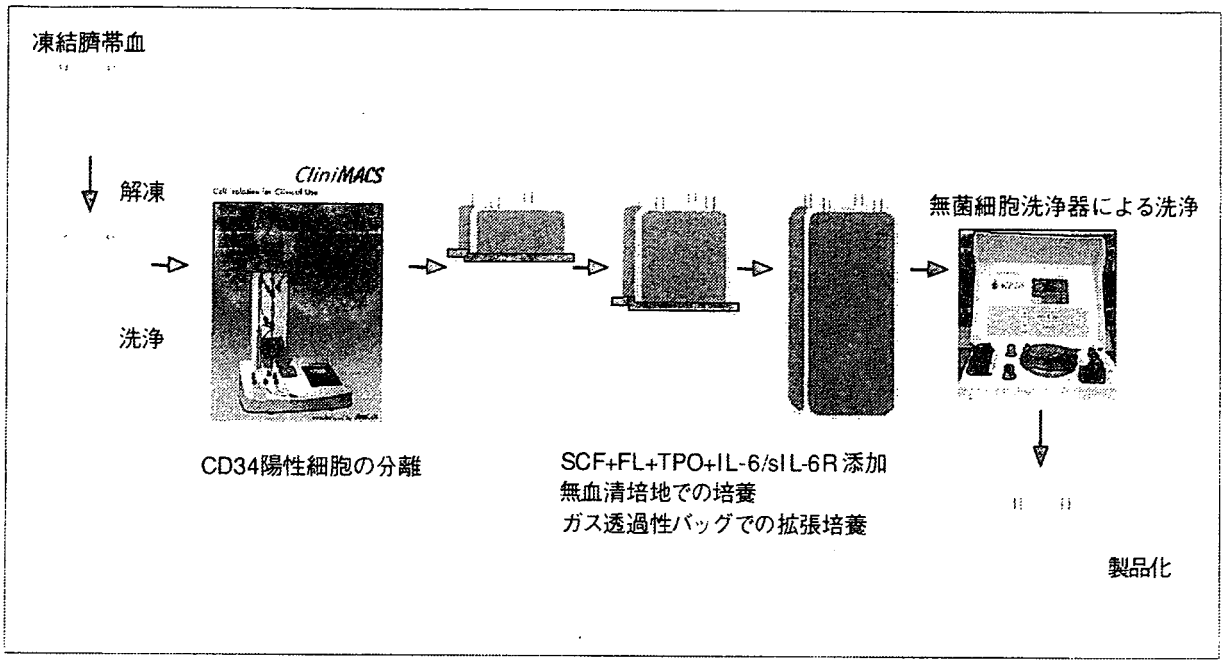

図3　GMP基準に準拠した*ex vivo*増幅臍帯血の製造工程

プが開始しており，いずれも増幅しない臍帯血と増幅した臍帯血を混合して移植する方法がとられている．2つの臨床試験の結果から明らかになったことは，*ex vivo*増幅臍帯血は安全に移植可能であり，生着不全率は低率であったが，期待されたような造血回復の促進効果は得られなかったということで，この結果は決して満足できるものではない．ただし両者の培養成績をみると，造血幹/前駆細胞が十分量移植されておらず，*ex vivo*増幅することの本来の目的である“より多くの造血幹/前駆細胞を移植すること”の効果が評価されていないと考えられる．一方Shpallらは，臍帯血より分離したCD133陽性細胞を銅キレート剤であるTEPA(tetraethylenepentamine)とサイトカインカクテルとを組み合わせた方法により21日間培養し，約200倍に増幅した細胞を移植した結果として，移植細胞数ならびに移植CD34陽性細胞数と好中球，血小板の生着期間との間で相関を認めたことを報告している[21]．今後症例数を増やした検討が期待される．また彼らは複数臍帯血移植において，一方を増幅した場合としなかった場合の比較試験を進めており，その結果も待たれる．

先端医療センターでの取り組み

われわれは先端医療センターでの*ex vivo*増幅臍帯血を用いた臨床研究を行うにあたり，平成14年度から京都大学，大阪大学，東京医科歯科大学，および各種企業との共同研究で以下のような総合的な整備を進めてきた．

1. GMP(good manufacturing practice)に準拠した培養法の確立

治療用細胞製剤を調整するためには，まず伝染物質の混入などを防止するための閉鎖系培養や無血清培養法の確立が必須である．われわれが開発した培養法は，凍結臍帯血からCD34陽性細胞を純化し，IL-6/sIL-6R, SCF, TPO, FL添加無血清培地にて12日間培養するもので，分離から培養，洗浄および最終製品出荷までのすべての工程を閉鎖系で行うことが可能である(図3)．

2. GMPに準拠した培養システムの開発，基盤整備

ハード面では先端医療センター内にCPC, Cell Processing Centerを整備すると同時に，個別型インキュベーターや無菌細胞洗浄装置などのデバイス開発を進めている．またソフト面では，作業手順書などの文書体系を作成し，安全な製造法，品質管理法を確立するための整備を行っている．

3. *Ex vivo*増幅した臍帯血幹細胞の品質管理法の確立

われわれは*ex vivo*増幅臍帯血の安全性および

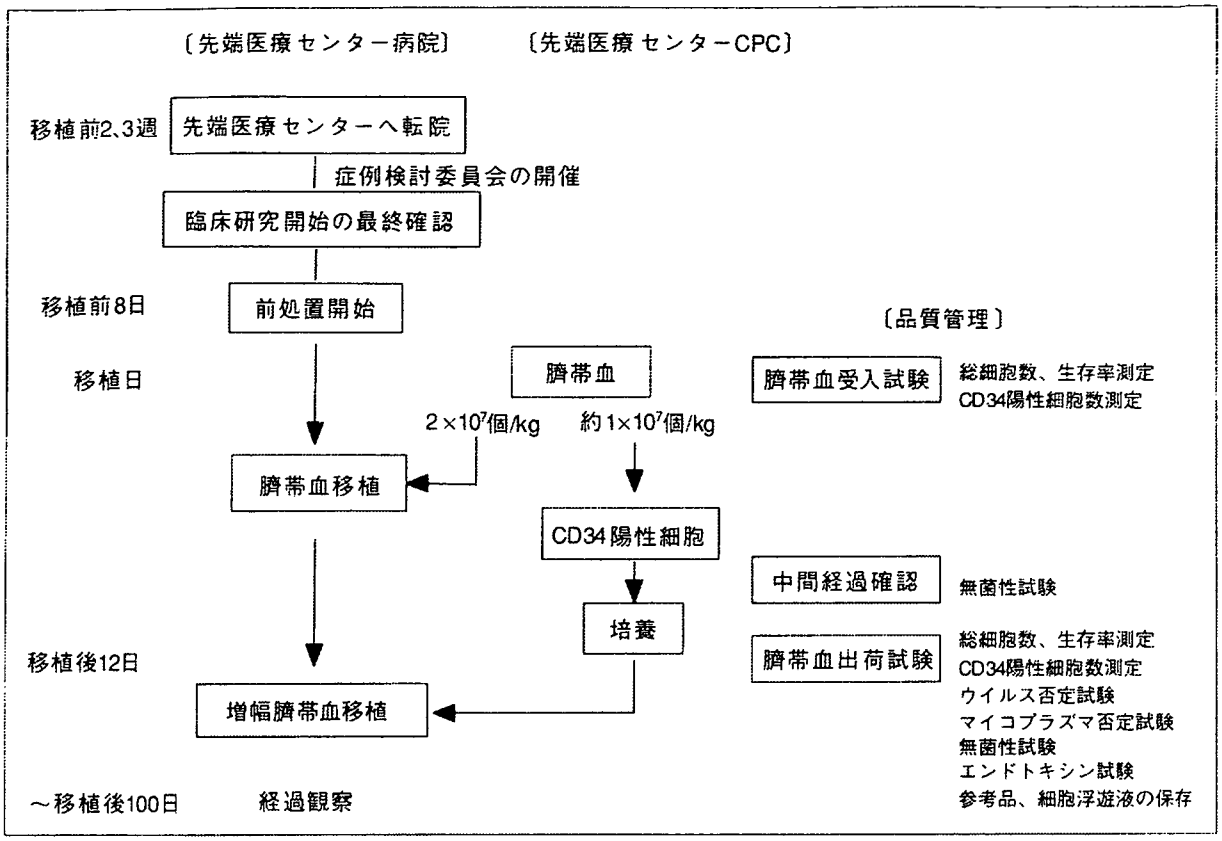

図4 GCPに準拠した*ex vivo*増幅臍帯血の臨床応用

性能について「ヒトまたは動物由来成分を原料として製造される医薬品等の品質および安全性確保について」(医薬発第1314号，厚労省医薬安全局長通知)に示されている概要に従う形で，*ex vivo*増幅臍帯血の性能および安全性を確認している．製造ごとに行う規格試験については，可能なかぎり最終製品の安全性，品質を担保すべく，製造工程に併せて実施できる体制をとっている(図4)．

4．臨床研究実施体制の確立

「トランスレーショナルリサーチ実施にあたっての共通倫理審査指針」[22]はトランスレーショナルリサーチの定義，理念およびその倫理審査体制についてまとめられたものであり，具体的な再生医療実践のための指針として2004年4月に発行された．また「ヒト幹細胞を用いる臨床研究に関する指針」はヒト幹細胞臨床研究が社会の理解を得て，適正に実施されるために2006年9月1日に厚生労働省より施行された指針であり，ヒト幹細胞臨床研究において，個人の尊厳と人権を尊重し，かつ科学的知見に基づいた有効性および安全性を確保するために，研究に携わるすべての者が遵守すべき事項が定められている．われわれは隣接する臨床研究情報センターの協力のもと，これら指針に従った形での臨床研究の実施体制を構築し，センター内倫理委員会，および日本さい帯血バンクネットワーク倫理委員会における承認を得た．

5．急性白血病患者に対する同種臍帯血由来*ex vivo*増幅CD34陽性細胞移植に関する臨床第I相/前期第II相試験

上述した基礎研究や基盤整備を背景として，現在われわれは，2006年4月より，*ex vivo*増幅臍帯血を用いた臨床研究を実施している．本臨床研究では日本造血細胞移植学会「造血幹細胞移植のガイドライン」の移植適応に合致し，骨髄移植および末梢血幹細胞移植において適切なドナーを得ることができない急性白血病の患者を対象としている．図4に示すように凍結臍帯血を解凍後に分割し，2.0×10^7/kg相当の臍帯血を未処理のまま移植，もう一方はCD34陽性細胞に純化後，上述した培養法で12日間増幅し移植するものであり，主要評価項目としてその安全性，さらには増幅培養したCD34陽性細胞の輸注

細胞数と生着率の相関を検討することを予定している．

今後の展望

近年の造血幹細胞に関する研究の結果，本稿で紹介したような臨床応用可能なレベルでの体外増幅法の開発が行われている．しかしながらその未分化性維持に関してはいまだ不明な点も数多く残されており，これらを解明することで，より効率のよいかつ安全な増幅方法の開発につながると考えられる．

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