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総　説

2. HTLV-1感染症とmiRNA

山岸　誠，渡邉　俊樹
東京大学大学院新領域創成科学研究科
メディカルゲノム専攻病態医療科学分野

HTLV-1はウイルス遺伝子産物によって感染T細胞を不死化，腫瘍化に導くが，成人T細胞白血病(ATL)を発症するまでの長い潜伏期間の背景にある分子機構は不明な点が多い．感染細胞及びATL細胞において様々な遺伝子発現異常が各々の特徴に寄与しているが，一方でそれらを制御する上流のイベントは不明な点が多かった．miRNAによる遺伝子発現調節という新たな概念が提唱されて以来，HTLV-1/ATLの領域においても複数の研究報告があり，分子レベルでの理解が深まったと言える．特に，ATLの多数の臨床検体を用いた網羅的解析の結果から，腫瘍細胞の特徴の1つであるNF-κBの恒常的活性化の分子メカニズムが明らかとなった．miRNAを介したエピジェネティック制御とNF-κB経路のクロストークも明らかとなり，miRNA研究から新たな分子機構も提唱された．一方でHTLV-1の生活環とmiRNAの関わりやmiRNA発現異常の原因解明など，今後の課題は多い．miRNAは多機能性であり，これらの分子基盤の創設がHTLV-1研究の今後の発展に寄与すると考えられる．

序論

microRNA (miRNA) は19－24merの非コードRNAで，標的遺伝子の3'UTRに結合し，RNAの分解と翻訳阻害を誘導する．標的遺伝子の認識には揺らぎがあるため，一つのmiRNAが複数の遺伝子発現に対して抑制的に働くことができる[1,2,3]．進化的にも保存性が高く，発生や分化，各細胞の機能において不可欠であることは十分に証明されている[1,4,5]．また疾患研究の成果から，miRNAの存在量のバランスが細胞の運命を支配していることも明らかである[6,7,8]．ウイルス学においてもmiRNA研究は盛んに進められており，新たなパラダイムを提供している．それは，外来種であるウイルスは，宿主細胞のmiRNAパターンに影響され，また逆にそのパターンに影響を与えるということである．つまり宿主側はウイルスに対する防御機構としてmiRNAを利用するし，一方でウイルスによるホストmiRNA発現への影響は，ウイルスによる病原性の本質の一つであることも示唆されている[7]．さらに，主にヘルペスウイルスにおいて，ウイルス自身がゲノム上にmiRNAをコードし，自身の生活環の維持と宿主に与える病原性に貢献することも報告されている[7,9,10]．短いRNAがウイルスと宿主の両者に与えるインパクトは，発見当初の予想を遥かに超えるものであったと言えるであろう．

HTLV-1研究領域におけるmiRNA研究は，未だ発展途上であり，十分に理解が進んでいないのが現状である．それは，HTLV-1がウイルス学的手法によって感染実験を進めることが極めて困難であり，HTLV-1とmiRNAの直接的な因果関係を証明することが容易でないことに起因する．しかし，HTLV-1感染によって樹立された細胞株や感染細胞の終末像である成人T細胞白血病（ATL）細胞の詳細な解析が行われ，HTLV-1に関連するmiRNAの全体像はクリアになりつつある．本総説では，HTLV-1/ATL研究領域の成果をまとめ，ウイルスとmiRNAの関係，及びmiRNA異常による分子病態を概説し，miRNA研究の

連絡先
〒108-8639
東京都港区白金台4-6-1
東京大学大学院新領域創成科学研究科
メディカルゲノム専攻病態医療科学分野
TEL: 03-5449-5298
FAX: 03-5449-5418
E-mail: myamagishi@mgs.k.u-tokyo.ac.jp
tnabe@ims.u-tokyo.ac.jp

今後の発展と課題について議論する.

HTLV-1 由来 miRNA の存在について

現在までに,EBV や KSHV などのヘルペスウイルス属を中心に,200 を超える多くのウイルス由来 miRNA が発見されている[7].これらの DNA ウイルスは,宿主 miRNA と同様にウイルス由来 miRNA を転写から Drosha − Dicer − RISC を経る miRNA の成熟過程をそのまま利用できる.また,ウイルス由来 miRNA は他のウイルスタンパク質と異なり短い RNA であるため免疫原性がなく,ウイルスの複製と潜伏化に適した環境を誘導することができる.さらに少しの変異が及ぼす影響が大きく,二本鎖 DNA の両方を効率よく利用できるという利点も,DNA ウイルスが miRNA をコードする理由であると考えられている.同様の事実はレトロウイルス属にも当てはまることであり,HIV-1 を中心に多くの研究者によって探索が行われている[10,11,12,13].HTLV-1 についてもウイルス由来 miRNA の存在が期待された.Li らはステムループ構造を予測するアルゴリズムを利用して HTLV-1 のセンス鎖に 3 つ,アンチセンス鎖に 8 つの miRNA を予測している[14].一方 Lin らは HTLV-1 感染細胞株 MT-2 細胞の 18-24 塩基の RNA から cDNA ライブラリを作成し,698 クローンのシークエンスを行ったが,HTLV-1 由来 miRNA は発見されなかったと報告している[13].Ruggero らは総説中でさらに詳細に解析したと言及しているが,やはり非常に限定的な検出しかされなかったと述べている[15].総合すると,HTLV-1 由来 miRNA は存在したとしても,限定された環境下でしかも非常に低レベルであることが想定される.ウイルス由来 miRNA は相補鎖のウイルス RNA に perfect match であり,ウイルスの複製効率とウイルス遺伝子による病原性に強く影響を与える.また多くの宿主遺伝子に対しても影響を及ぼすと考えられる.HTLV-1 が固有の miRNA を発現するか,またそれがどのような機能をもつのかという点は,生理的条件下における詳細な解析により,今後結論が出されるであろう.

HTLV-1 と宿主 miRNA の関係

他のウイルスと同様に,HTLV-1 も RNA として転写される際には複雑な 3' UTR 構造を持ち,宿主及びウイルス由来 miRNA の標的となる条件を満たしている.HIV-1 に関しては複数の研究グループによって宿主 miRNA によるウイルス RNA の抑制と潜伏化誘導が報告されている[16,17,18].この時,HIV-1 RNA は APOBEC3G とともに,P-body に存在することが示されている[18].一方 HTLV-1 については,アルゴリズムを用いた標的予測では複数の宿主 miRNA が HTLV-1 RNA に結合できることが予測されているが[15,19],実験的な検証は行われていない.今後,これらの miRNA 群の発現量とウイルスの複製レベルの関係を調べることにより明らかになるであろう.また,後述する網羅的な miRNA 発現解析の結果と統合することにより,有益な情報が得られると期待される.

一方,HTLV-1 が miRNA の合成経路に対して与える影響については少しずつ理解が進んでいる.Lin らは,宿主の持つ siRNA による標的遺伝子のノックダウン機構に対して,HTLV-1 Tax は大きな影響を与えなかったことから,Tax は RNAi の機構とはリンクしないとしている[13].一方 Abe らは,HTLV-1 Rex が Dicer と結合し,shRNA から siRNA への成熟過程に影響を与えることを報告している[20].またつい最近,Rahman らは Jurkat 細胞に Tax を導入した際の miRNA の発現パターンを網羅的に解析した[21].Tax を導入した細胞では 2 倍以上変化した 41 種の miRNA のうち,35 種の miRNA が減少していた.Tax が miRNA 合成経路に対してどのような分子機構で影響を与えるのか興味深い.また HTLV-1 は主に Tax を介して感染細胞のシグナル伝達系を撹乱することにより[22,23],miRNA の発現パターンにも大きく影響を与えると考えられる.miRNA の個別の機能が少しずつ明らかとなってきており,改めて HTLV-1 が宿主 miRNA に与える影響の解析が求められている.

HTLV-1 感染細胞における遺伝子発現異常と miRNA

標的遺伝子発現の恒常性の維持が miRNA の本来の機能の一つと言えるが,HTLV-1 感染細胞や ATL 腫瘍細胞では,その恒常性の破綻が明らかに証明されている.Cyclin-CDK 経路,p53 経路,JAK-STAT 経路などの異常の他に,特に NF-κB シグナルの著しい活性化が特徴的である[22,24].HTLV-1 感染細胞においてはウイルス遺伝子産物である Tax が NF-κB の定型的(canonical)及び非定型的(noncanonical)経路を強烈に活性化し,その結果,感染細胞はアポトーシス抵抗性を獲得する[22,23,25,26].一方で,ATL 細胞においては Tax の発現は非常に限定的であり,代わりに NF-κB の非定型的経路の上流に位置する NF-κB inducing kinase (NIK) が ATL 細胞において過剰に発現し,恒常的な NF-κB 経路の活性化を誘導する[27].現在までに様々な NF-κB 経路の阻害剤が開発され,いずれも感染細胞や ATL 細胞に対して強力なアポトーシスを誘導することから,感染細胞及び ATL 細胞は NF-κB 経路に "addict" した状態であると考えられる[28,29,30,31,32,33,34,35].また興味深い事に,HTLV-1 感染キャリアから分離した末梢血単核球 (PBMC) からは,通常 Tax の発現が認められないが,やはり NF-κB 経路が活性化しているデータが示された[28].このことからも NF-κB 経路の活性化は HTLV-1 感染症に対する重要な分子標的であると言える.NIK は ATL だけでなく,多発性骨髄腫やびまん性大細胞型 B 細胞リンパ腫 (DLBCL) などの造血器系腫瘍や,乳がんなどの固形がんにおいても重要な分子標的である[36,37,38,39].NIK の発現レベルは正常

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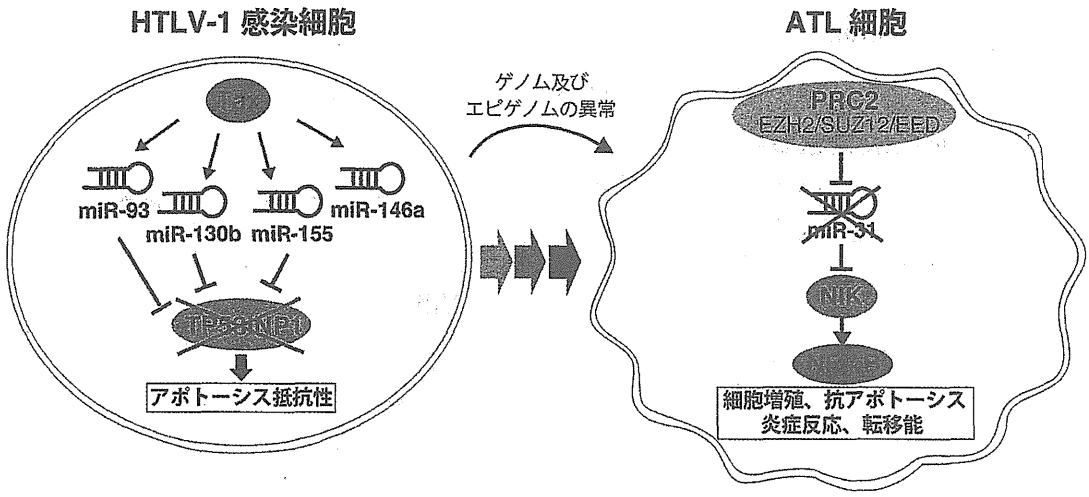

図1　HTLV-1感染細胞とATL細胞におけるmiRNAの異常
感染細胞ではTaxによって複数のmiRNAが発現誘導され，共通の標的遺伝子であるTP53INP1の発現が減少し，その結果アポトーシス抵抗性が獲得される．一方でATL細胞では，ゲノム及びエピゲノム異常の蓄積によってmiR-31の発現が例外なく欠損し，NIKの発現を介したNF-κB経路の恒常的活性化が誘導される．

細胞では主にタンパク質レベルで制御されているが[40]，ATL細胞におけるNIK mRNAの上昇や，他のがんにおけるNIK依存的なNF-κB経路の活性化メカニズムは明らかになっていなかった．

HTLV-1の感染が宿主のmiRNAパターンに与える直接的な影響は，正確に評価する実験系に高いハードルがある．HIV-1のように簡便で正確な感染実験系を構築するのが困難であり，またHTLV-1感染個体からリンパ球のごく一部に相当する感染細胞集団を濃縮する系も確立されていない．従ってHTLV-1感染によって樹立された細胞株による評価が行われている．

Pichlerらは，ATL及びHAM/TSP（HTLV-1関連脊髄症）患者由来細胞株，HTLV-1やTaxによってトランスフォームされた細胞株を用いてmiRNAの発現レベルを検討した[41]．検討したmiRNAはATL細胞の起源とされている制御性T細胞において重要なmiRNA群[42]，及び当時明らかにされていたがん関連miRNA群に限定している．その結果，HTLV-1関連細胞株でmiR-21，miR-24，miR-146a，miR-155が発現上昇し，miR-223が発現減少していることを明らかにした．またそのうちmiR-146aの過剰発現はTaxによるNF-κB経路の活性化が原因であることも明らかにしている．miR-146aはEBVのLMP1によるNF-κB経路の活性化によっても誘導されることが報告されている[43,44]．

Yeungらは，7種類のHTLV-1関連細胞株と4例の急性型ATL細胞を用いて327種のmiRNAの網羅的解析を行った[45]．対照群には正常のPBMCを用いている．彼らはさらにPMAによってPBMCを活性化させた際に上昇するmiRNA群で絞り込みを行い，HTLV-1感染とT細胞の活性化によって上昇するmiRNAとしてmiR-18a，miR-93，miR-130bを報告している．そのうち，miR-93とmiR-130bの共通する標的遺伝子としてTP53INP1を見いだし，HTLV-1感染細胞において，miR-93及びmiR-130bの過剰発現がTP53INP1の発現を低下させることにより，細胞生存の獲得に寄与していることを明らかにした．Pichlerらが報告したmiR-155はYeungらのATL細胞における過剰発現miRNAのリストにも含まれているが，実はその一年前にmiR-155が同じくTP53INP1を標的とすることが膵臓がんの研究から報告されている[46]．TP53INP1は様々な固形がんで癌抑制因子として注目されている[47,48,49,50]．またKSHVが発現するmiR-K12-11がmiR-155と高いホモロジーを持ち，miR-155の過剰発現と同様にB細胞の増殖をミミックするという興味深い報告もある[51]．HTLV-1感染細胞においては，複数のmiRNAで制御されるTP53INP1の発現低下が重要な役割を持つものと考えられる（**図1**）．miR-155の発現はB細胞株においてはLPSによって，前駆脂肪細胞においてはTNF-αによって，それぞれNF-κB依存的に発現が誘導されることが報告されている[52,53]．またmiR-130bのプロモーターにもNF-κB結合配列があり，上記のmiR-146aと同様に，TaxによってmiR-130bの発現が誘導される[45]．

ATLにおけるmiRNA異常

Yeungらは4例の臨床検体を用いることでATL腫瘍細胞におけるmiRNAについても検討している[45]．また2010年にBellonらは，同様に7例のATL検体について3例の正常PBMCもしくはCD4+ T細胞と比較を行っている[54]．その結果ATLではmiR-150，miR-155，miR-223，miR-142-3p，miR-142-5pが上昇し，miR-181a，miR-132，

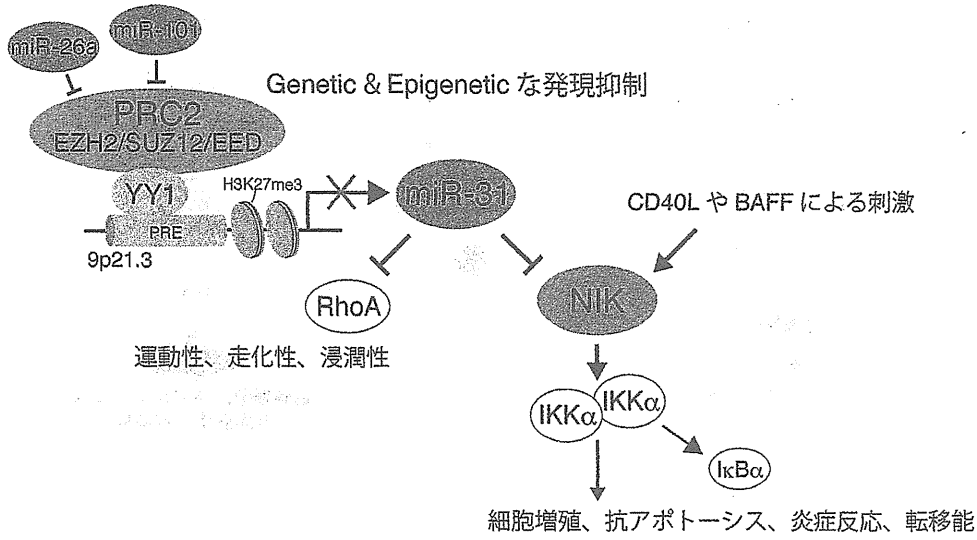

図2 ATL 細胞における miR-31 を取り巻く分子メカニズム
Polycomb 依存的な miR-31 の発現低下は NIK などの標的遺伝子を介して細胞の表現型に影響する．この分子間の関係は様々な細胞種で保存されており，各因子の存在量のバランスによって均衡が保たれている．バランスを崩した細胞は悪性化をたどると考えられる．

miR-125a，miR-146b が減少していると報告している．miR-155 の発現上昇は Pichler ら，Yeung らの報告と一致している．また興味深いことに，Yeung らと Bellon らはそれぞれ，患者由来 ATL 細胞と HTLV-1 によって樹立された細胞株は異なる miRNA 発現パターンを示すと言及している．

上記の3つの報告[41,45,54]では異常を示す miRNA パターンが多様であり，統一したデータが得られていない．その原因は，解析する症例数が少なく，異常を示す miRNA の絞り込みが甘いことが原因であると考えられる．また最近の知見から，リンパ球の各サブセットで miRNA の発現パターンが大きく異なることもわかっており[55,56]，ATL 細胞に対する正確な比較対象（=CD4+ T 細胞）を用意することが，ATL 細胞の異常を正確につかむことにつながると考えられる．また臨床検体については，腫瘍細胞集団と正常細胞の割合も結果に大きく影響するポイントである．

最近我々は，以上の問題に対して回答を得た．我々は HTLV-1 感染者コホート共同研究班 JSPFAD（http://www.htlv1.org/）の全面的協力を得て，世界で初めて ATL 患者由来腫瘍細胞の DNA, mRNA, miRNA の大規模な統合解析を完了した[57]．miRNA 解析のサンプルにはプロウイルス量の多い（＝腫瘍細胞の割合が高い）40 例の ATL 患者由来細胞を用い，さらにコントロール群には ATL 群と年齢を一致させた健常人 CD4+ T 細胞 22 例を用いた．アジレント社の 723 種のヒト miRNA と 76 種のウイルス由来 miRNA を網羅した microarray を用い，非常に厳しい検定をかけて異常 miRNA を割り出した結果，それまでに報告されていた上記の miRNA パターンと異なり，ATL では 61 種の異常 miRNA のうち 59 種の miRNA が正常 T 細胞に比べて著しく低値を示すことがわかった．これは，腫瘍細胞は miRNA の発現が低下傾向にあるという他のがん研究の結果と一致している[58,59]．減少している miRNA リストには，すでに癌抑制性 miRNA として報告されている Let-7 ファミリー[60]や miR-101[61]なども含まれていた．これらの 61 種の miRNA は ATL 細胞の新たな分子マーカーであり，また一つひとつが機能的に腫瘍細胞の特徴に寄与していると考えられる．我々のデータにおいても，上記の先の3つのグループによって報告されている miR-155 の発現上昇が示されていたが，厳しい統計的有意差（$p<0.00001$）は見られなかったため，リストから除外している（筆者ら，unpublished data）．

miR-31 の機能と発現欠損の分子メカニズム

ATL 細胞で見つかった 61 種の発現異常 miRNA のうち，miR-31 が例外なくすべての ATL 患者で減少し，且つ減少のレベルが著しいことに気がついた（0.00403 倍，$p = 2.85 \times 10^{-25}$）．miR-31 の発現減少は，乳がんにおける転移，浸潤過程において重要であることが報告されている[62,63]．我々は，miR-31 の著しい減少が ATL 細胞の特徴を反映していると考え，ATL 細胞の mRNA 発現プロファイルの結果と統合し，さらに *in vitro* の複数の実験を経て，miR-31 の新規標的遺伝子として，ATL 細胞における NF-κB 活性化の原因遺伝子である NIK を見いだした．実験の結果，正常 T 細胞では miR-31 の発現が比較的高く NIK の発現

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miRNA	標的遺伝子	参考文献
HTLV-1 感染細胞株		
上昇		
miR-155	TP53INP1	41,54
減少		
miR-150		45,54
miR-223		41,45,54
ATL 細胞		
上昇		
miR-150		45,54
miR-155	TP53INP1	45,54
減少		
miR-31	NIK, RhoA	45,57
miR-125a		54,57
miR-126		45,57
miR-130a		45,57
miR-146b		54,57
miR-181a		54,57
miR-355		45,57

図3　HTLV-1/ATL における miRNA 発現異常
HTLV-1 及び ATL 関連領域の研究のうち，複数の論文で報告されているものを示した．掲載した標的遺伝子は，それらの報告の中で実験的に検証されている遺伝子のみを示している．

を抑制しているが，miR-31 の異常な発現低下が NIK の発現誘導とそれに伴う NF-κB 経路の恒常的活性化を誘発することがわかった（**図1**）．さらに ATL 細胞株及び新鮮 ATL 細胞に対して miR-31 を再導入すると細胞死が誘導された[57]．このことは，miR-31 の細胞内レベルが腫瘍細胞の表現型に直接影響していることを意味し，新しい分子標的としての有用性が示された．

ATL 臨床検体を詳細に解析した結果，miR-31 の発現欠失はゲノムの欠損と Polycomb ファミリー依存的なエピジェネティックな異常によって，すべての ATL 患者で起こっていることがわかった．さらに，Polycomb ファミリーが miR-31 を抑制することによって NIK – NF-κB 経路を活性化する分子機構は，ATL だけでなく乳がん細胞や B 細胞における免疫応答反応においても保存されていることを明らかにした[57,64]．Polycomb ファミリー，NF-κB 経路，miR-31 はそれぞれが単独で多彩な機能を有しており[65,66,67,68,69,70,71,72,73]，細胞の恒常性や分化などの様々な機能に必須であると同時に，さらにクロストークを形成することによって，より複雑な遺伝子発現制御ネットワークに昇華させていると考えられる（**図2**）．我々の実験結果は，ATL 細胞はこの分子ネットワークに依存していることを示しており，エピジェネティックの制御，もしくは miR-31 の補充による新たな治療法の開発につながると期待される．

今後の展望

複数の研究グループによって詳細に解析され[41,45,54,57]，HTLV-1 感染細胞及び ATL 腫瘍細胞の miRNA 発現パターンはほぼ明らかになった（**図3**）．しかし，実際に異常 miRNA の機能とその影響を詳しく解析されているのは miR-93，miR-130b，miR-155 及び miR-31 だけである．標的遺伝子として明らかになった TP53INP1 や NIK にとどまらず，個々の miRNA とその標的遺伝子が感染細胞や腫瘍細胞に対してどのような役割を持つかが，今後の研究課題である．miRNA の標的遺伝子は複数のアルゴリズムによって予測可能であるが，物理的な抑制効果の検証と，標的遺伝子側の機能や挙動も重要な指標となり，従って多角的な実験的検証が必須となることを特筆しておく．

一方で，HTLV-1 と miRNA の研究はまだ始まったばかりである．残っている疑問点は以下に挙げられる．① HTLV-1 が宿主 miRNA システムに与える影響について．感染によって樹立された細胞株からの情報だけでなく，感染というイベントが宿主 miRNA に与えるインパクトについて詳細に検討する必要がある．特に Tax，Rex，HBZ などのウイルス因子が miRNA の合成経路に対してどのような影響を与えるのかは今後の課題である．Rahman らが報告した Tax による宿主 miRNA に対する全体的な抑制効果

は[21]，我々が明らかにしたATLにおけるmiRNAの全体的な減少[57]とリンクするのか，今後の展開が待たれる．②HTLV-1 RNAが宿主もしくはHTLV-1由来miRNAの標的となるのか，実験的検証が必要である．近年になってHTLV-1の伝播様式やレセプターが少しずつ明らかとなっており[74,75,76,77]，またHTLV-1やTaxによる病原性がin vivoにおいても様々なステージにおいて証明され始めている[78,79,80]．様々な環境下におけるHTLV-1の生活環に対してmiRNAがどのような影響を与えるのか，重要な研究課題である．③HTLV-1由来miRNAの存在について．上述したように，ヘルペスウイルスのような高発現miRNAはHTLV-1には存在しないと考えられるが，次世代シークエンサ等の技術導入により明らかにされると期待される．④HTLV-1感染細胞からATL細胞のmiRNAパターンの変遷について．感染細胞はTaxによるNF-κB経路の活性化が良く反映されている一方[45]，終末像であるATLのmiRNAの発現異常は非常に特徴的であり，ゲノム及びエピゲノムの異常によって誘導されるmiRNAパターンにaddictしていると考えられる[57]．どのような分子機構でATL細胞が形成されていくか，エピジェネティックやmiRNA合成経路の詳細な解析が必要である．⑤HTLV-1に関連する他の疾患とmiRNAについて．HAM/TSPやHTLV-1関連ぶどう膜炎（HU）などの疾患についてはmiRNAの検討は行われていない．各疾患の分子病態を理解する上でmiRNAの網羅的な検討は必須である．また感染キャリアにおけるmiRNAパターンを把握することで，発症の分子メカニズムの一端が明らかにされるであろう．

おわりに

1993年に線虫で初めてmiRNAが発見されて以来[81,82]，急速に研究が進展し，miRNAに関する知識も深まってきた．miRNAは遺伝子発現全体を制御する上流の分子群であり，ウイルス感染症を考える上でも欠かす事のできない因子として位置づけられている．ウイルス学のように生理学的意義を検討する学問においては，予測や他の報告だけに頼らず，実験的検証が正しい情報発信に重要である．HTLV-1感染症の分子レベルの理解と治療法の開発を目指す上で，miRNA研究の推進は急務であると考えられる．

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Online reporting system for transfusion-related adverse events to enhance recipient haemovigilance in Japan: A pilot study

Chikako Odaka [a], Hidefumi Kato [b], Hiroko Otsubo [a], Shigeru Takamoto [b], Yoshiaki Okada [a], Maiko Taneichi [a], Kazu Okuma [a], Kimitaka Sagawa [c], Yasutaka Hoshi [d], Tetsunori Tasaki [d], Yasuhiko Fujii [e], Yuji Yonemura [f], Noriaki Iwao [g], Asashi Tanaka [h], Hitoshi Okazaki [i], Shun-ya Momose [j], Junichi Kitazawa [k], Hiroshi Mori [l], Akio Matsushita [m], Hisako Nomura [n], Hitoshi Yasoshima [o], Yasushi Ohkusa [p], Kazunari Yamaguchi [a], Isao Hamaguchi [a,*]

[a] *Department of Safety Research on Blood and Biological Products, National Institute of Infectious Diseases, Tokyo, Japan*
[b] *Department of Transfusion Medicine, Aichi Medical University, Aichi, Japan*
[c] *Department of Laboratory Medicine, Kurume University, Fukuoka, Japan*
[d] *Department of Transfusion Service, Tokyo Jikei University, Tokyo, Japan*
[e] *Department of Blood Transfusion, Yamaguchi University School of Medicine, Yamaguchi, Japan*
[f] *Department of Transfusion Medicine and Cell Therapy, Kumamoto University, Kumamoto, Japan*
[g] *Department of Transfusion Medicine and Cell Therapy, University of Yamanashi, Kumamoto, Japan*
[h] *Department of Blood Transfusion, Tokyo Medical University Hachioji Medical Center, Tokyo, Japan*
[i] *Central Blood Institute, Blood Service Headquarters, Japanese Red Cross Society, Tokyo, Japan*
[j] *Blood Service Headquarters, Japanese Red Cross Society, Tokyo, Japan*
[k] *Kuroishi General Hospital, Aomori, Japan*
[l] *Minami Tama Hospital, Tokyo, Japan*
[m] *Shibetsu City Hospital, Hokkaido, Japan*
[n] *Sanraku Hospital, Tokyo, Japan*
[o] *Yao General Hospital, Osaka, Japan*
[p] *Infectious Diseases Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan*

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ABSTRACT

Background: A surveillance system for transfusion-related adverse reactions and infectious diseases in Japan was started at a national level in 1993, but current reporting of events in recipients is performed on a voluntary basis. A reporting system which can collect information on all transfusion-related events in recipients is required in Japan.
Methods: We have developed an online reporting system for transfusion-related events and performed a pilot study in 12 hospitals from 2007 to 2010.
Results: The overall incidence of adverse events per transfusion bag was 1.47%. Platelet concentrates gave rise to statistically more adverse events (4.16%) than red blood cells (0.66%) and fresh-frozen plasma (0.93%). In addition, we found that the incidence of adverse events varied between hospitals according to their size and patient characteristics.
Conclusion: This online reporting system is useful for collection and analysis of actual adverse events in recipients of blood transfusions and may contribute to enhancement of the existing surveillance system for recipients in Japan.

* Corresponding author. Address: Department of Safety Research on Blood and Biological Products, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan. Tel.: +81 42 848 7120; fax: +81 42 567 2790.
E-mail address: 130hama@nih.go.jp (I. Hamaguchi).

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1. Introduction

Haemovigilance is defined as the surveillance of transfusion-related adverse reactions occurring in donors and in recipients. The ultimate purpose of haemovigilance is to prevent adverse events caused by blood products to ensure maximum safety. Various haemovigilance systems have been implemented around the world, with a different approach in different countries [1–6].

In Japan, the Japanese Red Cross Society (JRCS) is the sole provider of labile blood products, and controls blood collection, processing and supply nationwide. The JRCS, in cooperation with the national government, has been collecting data on transfusion-related adverse reactions and infections nationwide since January 1993 [7]. Epidemiological surveillance in donors is being performed to ensure their health as well as the safety and quality of blood components. For recipients, suspected adverse reactions, including infections related to the blood products, are reported from medical institutions to the JRCS on a voluntary basis, and nearly 2000 suspected cases were reported each year from 2004 to 2008 [7]. The JRCS investigates the relationship between transfusion and the reported adverse events. Based on the analysis, the JRCS evaluates blood safety with the government to take appropriate and immediate measures, as required, in JRC blood centers and medical institutions. The existing surveillance system for recipients has functioned well over a number of years, and most of the reported cases have been relatively moderate to severe. However, comprehensive data on adverse transfusion reactions in all recipients are unavailable. We therefore need to establish an improved system for monitoring recipients nationwide.

We have developed an alternative reporting system to collect data on all transfusion-related reactions in recipients. A pilot study of this online surveillance system has been performed since January 2007. Here, we describe our online system and present the data collected by 12 medical institutions from January 2007 to December 2010.

2. Materials and methods

2.1. Participants in the pilot study

Seven university hospitals (Aichi Medical University, 1014 beds; Tokyo Jikei University, 1075 beds; Yamanashi University, 600 beds, Tokyo Medical University Hachioji Medical Center, 621 beds; Yamaguchi University, 759 beds; Kurume University, 1186 beds; Kumamoto University, 843 beds) initially participated in the pilot study in 2007, and five small-scale hospitals with fewer than 300 beds (Kuroishi General Hospital, Minami Tama Hospital, Shibetsu City Hospital, Sanraku Hospital, Yao General Hospital) joined this study 2 years later.

2.2. Online system

In the participating hospitals, doctors or nurses monitored transfusion-related reactions at 0, 5, and 15 min after starting transfusion, at the end of transfusion, and within 6 h after finishing the transfusion. Severe adverse events and infections were determined after detailed diagnosis in JRC blood centers. These data were gathered in the hospital transfusion department. Doctors or transfusion specialists in the department reported the data every 2 months via the worldwide web (https://www.1597532.net/). Data were collected in the National Institute of Infectious Diseases, and analyzed statistically every 2 months. The online surveillance system was password-protected, and respondents were provided with an identification and password.

2.3. Statistics

All statistical analyses were performed by the Student *t* test. Probability values less than 0.05 were considered statistically significant.

3. Results

3.1. Reporting system and classifications

Our online surveillance system was designed to collect all transfusion-related reactions in recipients. The system monitored the total number of transfusions of three types of labile blood component: red blood cells (RBC), platelet concentrates (PC) and fresh-frozen plasma (FPP), in each reporting period (Fig. 1). The number of transfusion reactions, and clinical signs and symptoms were also collected. They were classified into 16 categories, as shown in Fig. 2. Additionally, information on diagnostic data was collected (Fig. 3). Transfusion-related adverse events were categorized into non-haemolytic reactions, haemolytic reactions and post-transfusion infectious diseases. The non-haemolytic reactions included: severe allergic reaction, transfusion-related acute lung injury (TRALI), transfusion associated circulatory overload (TACO), post-transfusion purpura (PTP) and transfusion-associated graft-versus-host disease (TA–GVHD). Definitions of these severe transfusion reactions were in accord with the International Society of Blood Transfusion [8]. For non-haemolytic reactions or infections, those events not covered by the diagnoses listed were assigned to the category "Others".

3.2. Number and frequency of adverse events from 2007 to 2010

We investigated transfusion reactions collected by 12 hospitals from January 2007 to end of December 2010 (Fig. 4). During the period, 241,225 bags of labile blood products were used in 12 hospitals: 133,993 bags of RBC, 55,861 bags of FFP and 51,371 bags of PC (Fig. 4B). The proportions of RBC, FFP and PC were 55.5%, 23.2% and 21.3%, respectively, of the total amount of blood bags (Fig. 4A). There were 3,539 transfusion-related adverse events reported during the period (Fig. 4B). Of the reported reactions, the blood product that accounted for highest proportion of adverse events was PC (60.4%), followed by RBC (24.9%) and FFP (14.7%) (Fig. 4A). When the frequency of transfusion reactions was calculated according to the total number of bags, the overall incidence of adverse events was 1.47% (Fig. 4B). PC was found to induce transfusion reactions at a

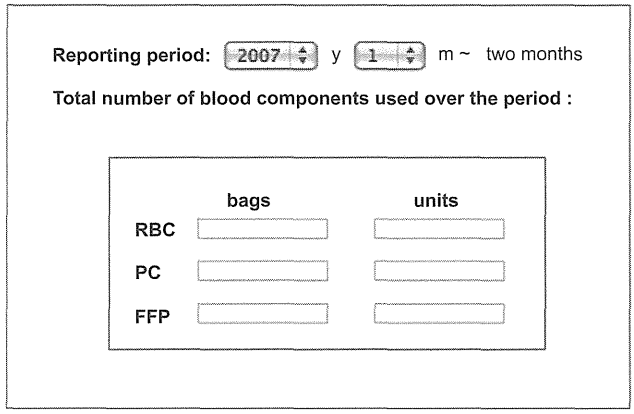


Fig. 1. Online surveillance system (1): Screenshot of the total number of the three labile blood components (bags and units) used over each reporting period. RBC: red blood cells; FFP: fresh frozen plasma; PC: platelet concentrates.

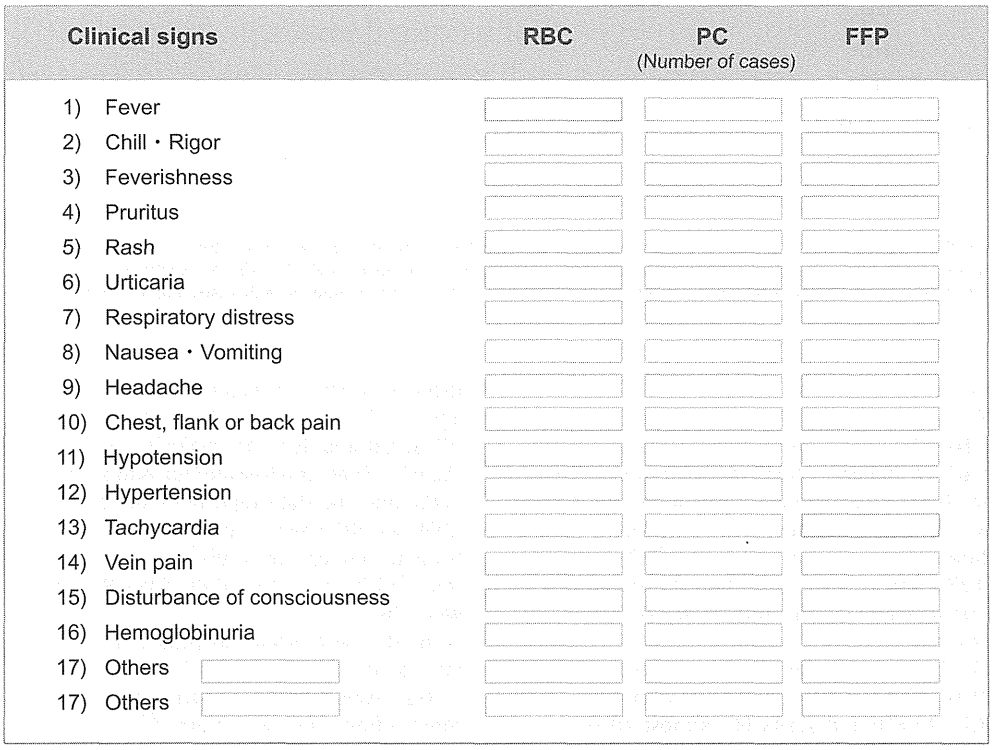


Fig. 2. Online surveillance system (2): The total number of transfusion reactions by clinical signs for the three blood components used over the reporting period is presented. Clinical signs are classified into the 16 categories indicated. Fever: more than 38 °C or a 1 °C or more increase from the baseline; hypotension: a decrease of more than 30 mmHg from the baseline; hypertension: an increase of more than 30 mmHg from the baseline; tachycardia: more than 100 times/min for adult, modified according to age for children. Any findings other than the 16 signs can be entered as free text in "Others".

rate of 4.16%. The incidence of transfusion reactions with RBC and FFP was 0.66% and 0.93%, respectively. The annual incidence of adverse events showed a similar tendency (RBC < FFP < PC) every year, as shown in Fig. 4C.

3.3. Types, clinical signs and diagnoses of adverse events

Next, we analyzed the types, clinical signs and diagnoses of adverse events collected from 12 hospitals over 4 years. The types of adverse events among the different blood components were diverse (Fig. 5A). Febrile non-haemolytic transfusion reactions (FNHTR) were more often found with RBC than with FFP or PC. Allergic reactions were observed significantly more often with FFP or PC than with RBC. In the reactions to RBC, 36.6% were FNHTR and 31.2% were caused by allergic reactions. Respiratory distress, a hypotensive reaction, and a hypertensive reaction accounted for 3.9%, 8.0% and 4.4%,

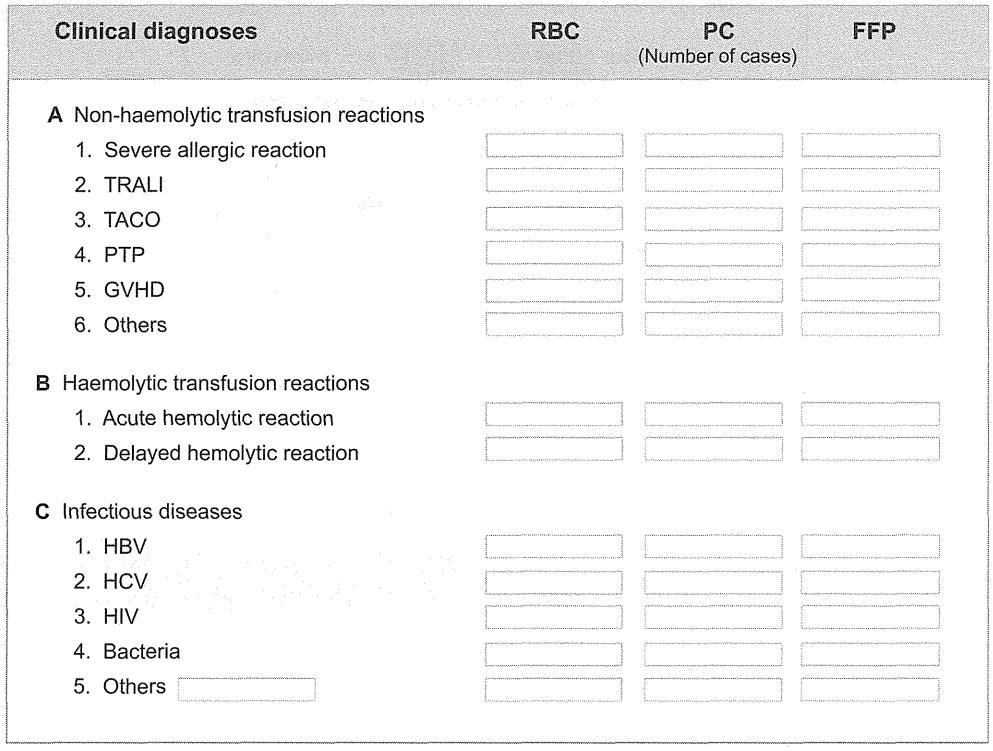


Fig. 3. Online surveillance system (3): The total number of transfusion reactions by clinical diagnoses for the three blood components over the period is presented. Clinical diagnoses are classified into the three categories indicated. Among non-haemolytic transfusion reactions, the events not included in the diagnoses listed are placed in the category "Others". For infections, any findings other than the infectious diseases indicated can be entered as free text in "Others".

respectively, of the transfusion-related events. For PC, more than 80% of the reactions were allergic and 11.6% were FNHTR. For FFP, 70.8% were allergic reactions. The clinical signs of transfusion reactions were assessed by the events per bag of each blood component (Fig. 5B). In the reactions to RBC, fever occurred in 0.2% of transfusion bags, followed by urticaria in 0.15%. In FFP, pruritus occurred in 0.23% and urticaria in 0.54%. PC induced fever, pruritus or urticaria at the rate of 0.32%, 0.98% or 2.85%, respectively.

As shown in Fig. 4B and Table 1, 3,539 reaction events were collected during the 4-year period, of which 881 were caused by RBC, 520 FFP and 2,138 PC. Almost all the adverse reactions reported were "Others" in non-haemolytic reactions. Severe allergic reaction, TRALI or TACO were reported at the rate of 0.1–1.3% for each blood component. In the adverse events for RBC, four cases of hemolytic reactions and one case of HBV infection were reported.

3.4. Variation in the incidence of adverse events by medical institutions

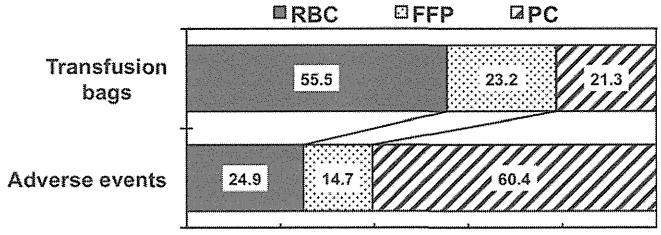
We compared the incidence of adverse events in seven large-scale university hospitals with that in five small-scale hospitals with fewer than 300 beds. Seven large-scale hospitals participated in this pilot study since 2007 and the data reported by these hospitals from 2007 to 2010 were analyzed (Fig. 6A). A total of 231,662 transfusion bags were used, of which over half were RBC, followed by FFP (23.6%) and PC (21.9%). Among the 3,410 adverse events reported, PC accounted for the majority of transfusion reactions (62.6%). Five small-scale hospitals joined this study in 2009, and the data reported from these institutions from 2009 to 2010 were analyzed (Fig. 6B). A total of 9,563 transfusion bags were used and 129 adverse events were reported in these hospitals. Over 80% of transfusion bags were RBC.

In the large-scale hospitals, the incidence of adverse events per bag of RBC, FFP or PC was 0.61%, 0.94% and 4.20%, respectively, indicating that adverse events were more often observed with PC than with FFP or RBC (Fig. 6C). On the other hand, in the small-scale hospitals, the incidence of adverse events per bag of RBC, FFP or PC was 1.46%, 0.98% and 0.59%, respectively, indicating that the adverse events were more often observed with RBC than with PC or FFP (Fig. 6C). There was a significant statistical difference in the incidence of transfusion-related adverse reactions per bag of RBC or PC in the large-scale vs. the small-scale hospitals.

4. Discussion

In our new reporting system, we analyzed the data collected from 12 medical institutions from 2007 to 2010. During the period, 241,225 labile blood products were used in these hospitals. Considering the number of blood

A. Rates of transfusion bags and adverse events by kinds of blood components



B. Incidence of transfusion reactions by kinds of blood components

	RBC	FFP	PC	Total
No. of transfusion bags	133,993	55,861	51,371	241,225
No. of adverse events	881	520	2,138	3,539
Incidence (%)	0.66	0.93	4.16	1.47

C. Annual incidence of adverse events by kinds of blood components

Year	RBC (%)	FFP (%)	PC (%)	Total (%)
2007	0.54	0.63	3.44	1.16
2008	0.61	0.69	4.22	1.45
2009	0.79	1.19	5.36	1.91
2010	0.70	1.30	3.77	1.49

Fig. 4. Proportions of transfusion bags and adverse events from 2007 to 2010. (A) The proportion of transfusion bags for each blood component and the proportion of adverse events ascribed to each component. (B) The incidence of transfusion reactions by type of blood component. (C) The annual incidence of adverse events by type of blood component.

products distributed nationwide during the 4 years, we monitored approximately 1% of the bags distributed in Japan for each blood component (data not shown). During this time, 3,539 transfusion-related adverse events were reported in this system, and the overall incidence of adverse events per bag was 1.47%. This incidence was higher than the reports from other countries which had 2.2–4.2 events per 1,000 blood products distributed [9–12]. We observed that the rate of reported cases varied considerably among seven university hospitals (data not shown). The true incidence of adverse events may be obscured by misdiagnosis. The lack of agreed definitions negatively affects data collection. The difficulty in the diagnosis of transfusion reactions also leads to misreporting. Therefore, sharing diagnostic criteria for transfusion-related reactions is required. Other studies in Japan have demonstrated similar incidences of adverse events by type of blood component (Kurata Y. et al., personal communication, 2007). Therefore, it is likely that our results reflect the real incidence of adverse events for blood products distributed in Japan.

PC (4.16%) gave rise to statistically more adverse events (6-fold) than RBC (0.66%) and FFP (0.93%). Our results were concordant with a previous report in Switzerland [12], although it should be noted that all products of PC in Japan are from single apheresis donor. PC was found to frequently induce fever, pruritus or urticaria. PC recipients, most of whom suffer from hematological diseases, tend to receive frequent blood transfusions. The repeated alloimmunization with PC may induce a high incidence of adverse events. We found that the incidence of adverse events varied between the university hospitals and the small-scale hospitals, based on the number of beds and patient characteristics. In Japan, most patients with hematological diseases have a check-up in large-scale hospitals including university hospitals. Actually, the five small-scale hospitals had no patients with hematological diseases, and their incidence of adverse events to PC was only 0.59%.

This online reporting system makes it possible to collect all transfusion-related adverse events in recipients rapidly. The database can perform calculations on the reported information automatically, and the results, such as the total number of adverse events or the incidence of adverse events, are fed back to participants continuously. This feedback should contribute to improving the safety of transfusion therapy in each medical institution. There are

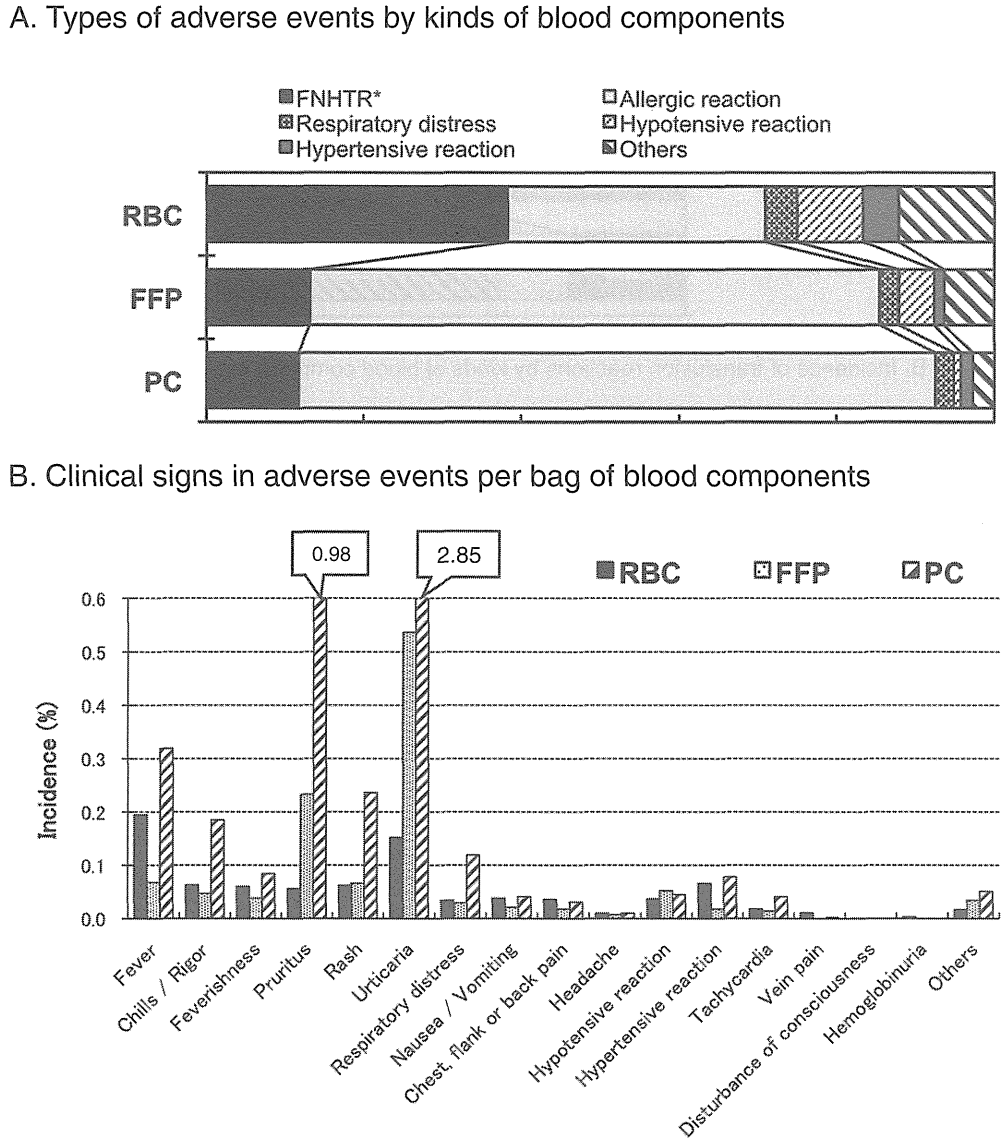


Fig. 5. Types of adverse events and clinical signs of adverse events by blood component. (A) Proportions of adverse events by type of blood component. (B) Incidence of clinical signs of adverse events by type of blood component. FNHTR: febrile non-haemolytic transfusion reaction.

a few limitations in this system. The focus of our study was only on three types of labile blood components. Information about the appearance of antibodies for each blood product was not collected. In addition, reporting of information on transfusion errors, including incorrect blood component transfusion and near-miss events, was out of the scope of the system. Almost all the adverse reactions collected for 4 years were "Others" in non-haemolytic reactions. As regards the severity of transfusion-related reactions, we speculated that the majority reactions were relatively mild. We did not confirm the individual cases of serious adverse events in this system during the period of the pilot study.

In the future, more detailed analyses of data collected by this system will be needed to determine how to improve the transfusion service and formulate new strategies to reduce adverse transfusion reactions. Almost all European Union countries have established a haemovigilance system and the number of haemovigilance systems outside Europe is steadily increasing. National haemovigilance systems linked to an international network will be indispensable to ensure the safety and quality of blood transfusions. Thus, an international standardized and centralized method for data collection and reporting is required. We have to continue to carefully monitor and compare the incidence of adverse events between Japan and other countries, in order to promote preventive measures in the manufacture of blood products in Japan, and other necessary steps to reduce transfusion-related events.

Table 1
Clinical diagnosis of transfusion-related adverse events from 2007 to 2010.

	RBC cases (%)	FFP cases (%)	PC cases (%)
Non-haemolytic transfusion reaction			
Severe allergic reaction	4 (0.5%)	7 (1.3%)	8 (0.4%)
TRALI	4 (0.5%)	3 (0.6%)	3 (0.1%)
TACO	4 (0.5%)	1 (0.2%)	0
PTP	0	0	0
GVHD	0	0	0
Others	861 (97.7%)	509 (97.9%)	2127 (99.5%)
Haemolytic transfusion reaction			
Acute hemolytic reaction	3 (0.3%)	0	0
Delayed hemolytic reaction	1 (0.1%)	0	0
Infectious diseases			
HBV	1 (0.1%)	0	0
HCV	0	0	0
HIV	0	0	0
Bacteria	0	0	0
Others	0	0	0
Total all cases	881	520	2138

The number of events and their frequency for each blood component are shown. TRALI, transfusion-related acute lung injury; TACO, transfusion associated circulatory overload; PTP, transfusion purpura; GVHD, graft-versus-host disease; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

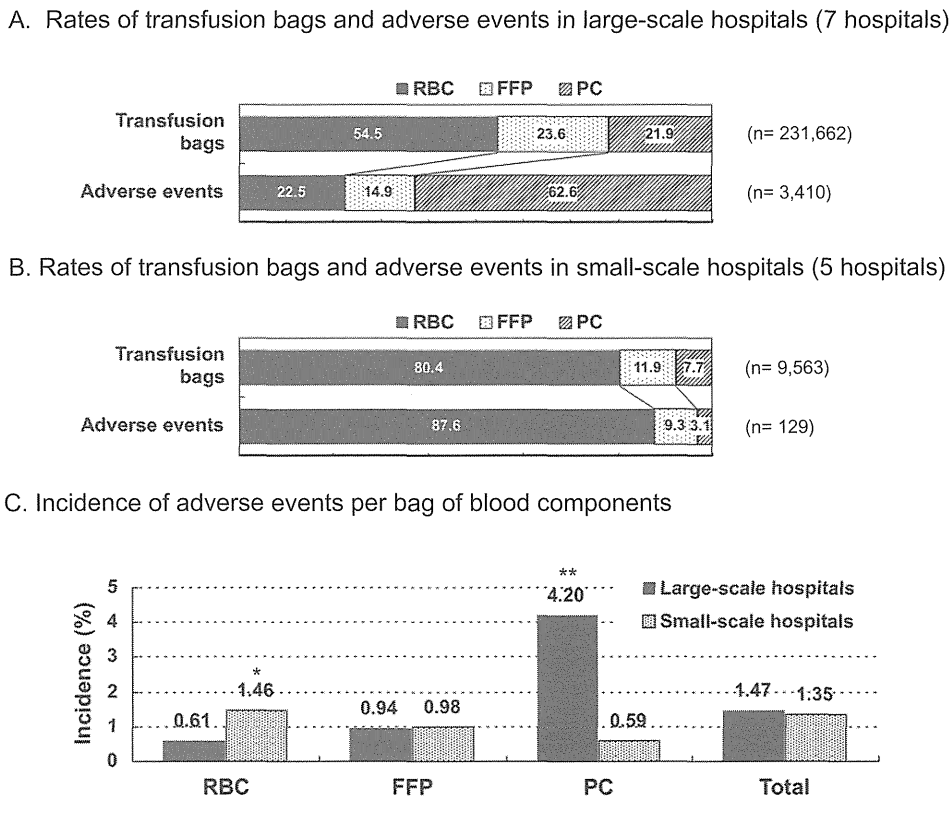


Fig. 6. Comparison of use of transfusion bag type, adverse events and incidence between large-scale and small-scale hospitals. Proportions of type of blood component and adverse events by type of blood component in seven large-scale university hospitals (A) and in five small-scale hospitals (fewer than 300 beds) (B). (C) The incidence of adverse events per bag of each blood component in large-scale and small-scale hospitals. $^{*}p < 0.05$ compared with large-scale hospitals; $^{**}p < 0.01$ compared with small-scale hospitals.

5. Conclusions

We have developed a comprehensive online system for the collection of all adverse reactions in recipients related to blood transfusion. Despite the limitation of our current system described above, this system is effective for collection and analysis of actual adverse events in recipients and can be used to enhance the existing surveillance system in Japan.

Conflict of interest statement

The authors declare no competing financial interests.

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