に，これら抑制活性を示したshRNAを導入した細胞から9クローンを選び，CD4の細胞表面発現が有意に低下していないことも確認した。また，この9クローン中7クローンについてはHIV-1複製抑制のメカニズムについて予備的実験を行い，HIV-1の逆転写，転写，Gag蛋白質の細胞内輸送に影響を与えるものも見出した。このスクリーニング方法の欠点としては，特長としても記載したshRNAによるノックダウンが細胞毒性を示す遺伝子を排除してしまうことで，完全な（または長期的な）ノックダウンが細胞毒性を示す宿主因子の中にHIV-1複製に必要な（もくしは抑制的に作用する）因子がある場合，そのような因子を取り逃してしまう可能性がある。

以上，HIV-1複製に必要な宿主因子を探索・同定するために行われてきた4つのスクリーニングを紹介してきた。各スクリーニング法の比較を表1に示した。いずれのスクリーニング結果もHIV-1複製に必要な宿主因子に関して有益もしくは新たな情報を提供したが，スクリーニング間でオーバーラップした因子の少なさに見られるように，得られる候補遺伝子は種々の実験条件や結果の選択法によって大きく変動すると考えられ，さらなる方法の改良が必要と考えられる[7]。shRNAスクリーニングに関していえば，癌研究の分野で試みられている誘導性のshRNAベクターの使用によって通常のshRNA導入法ではノックダウンによる細胞毒性によって排除してしまう宿主因子についても評価することができると期待される[8]。また，応用面では"Synthetic lethal"と呼ばれる通常のsiRNAスクリーニングと薬剤処理を組み合わせた方法がある。すなわち，ある特定の遺伝子をノックダウンすることによってごく低濃度の薬剤を併用することによって高濃度の薬剤処理と同等の効果を得ることに成功している[9]。この方法はすでに使用されている抗HIV剤や毒性が高くて使用できなかった薬剤についても適用できるかもしれない。

HIV複製を制御する宿主因子を探索するためのその他の方法

1. 機能的ゲノムワイドHIV耐性遺伝子スクリーニング

siRNA（shRNA）によるスクリーニングは主にHIV複製に必要な宿主因子の探索・同定の方法であるが，これとは対照的に主にHIV複製に耐性を示す宿主因子の探索・同定に用いられている方法である。レンチウイルスベクターに組込んだcDNAライブラリーをT細胞株に導入・安定発現させ，HIV-1感染後生存した細胞からHIV-1耐性遺伝子を同定する[10]。これまで，この方法論でCD63のN末欠損変異体がHIV-1のコレセプターの一つCXCR4の形質膜への輸送を阻害すること[11]や，Brd4のC末端領域がTatに依存したHIV-1 LTRからの転写を抑制すること[12]などが明らかにされた。

2. HIV感染・非感染細胞を用いた遺伝子発現プロファイルの網羅的解析

この方法論は，HIV感染によって特異的にその発現が上昇（もしくは減少）する遺伝子を同定し，それらをHIV複製に関与する宿主因子同定のためのプローブとして研究を進めようという試みである[13-15]。この試みから最近SOCS1とよばれるサイトカインのシグナル伝達に関わることが知られている蛋白質がHIV-1 Gag蛋白質の細胞内輸送とその安定性に寄与していることが明らかにされ，まだ十分に解明されていないHIV-1の粒子形成過程に貴重な知見を付け加えた[16,17]。

3. HIV感染・非感染細胞またはウイルス粒子を用いた蛋白質発現プロファイルの網羅的解析

このアプローチもやはり，HIV感染によって特異的にその発現が上昇（もしくは減少）する遺伝子産物（蛋白質）またはHIV粒子に取り込まれている宿主因子を同定し，それらをHIV複製に関与する宿主因子同定のためのプローブとして研究を進めようという試みである[18-21]。ウイルス感染によって特異的にその発現が上昇（もしくは減

表1 ゲノムワイドなsiRNA（shRNA）スクリーニングの比較

スクリーニング	標的細胞	ウイルス	siRNA処理時間（h）	抗ウイルス活性の判定時間（h）	ウイルス複製のパラメーター
Brass et al.	HeLa-CD4 CCR5 tat-β-gal	HIV-1 IIIB	72	48 48（新規感染）	p24 (CA) β-galtosidase活性
König et al.	293T	VSV-Gシュードタイプ HIV-1 Luciferase	48	24	luciferase活性
Zhou et al.	HeLa-CD4 CCR5 tat-β-gal	HIV-1 HXB2	24	48/96	β-galtosidase活性
Yeung et al.	Jurkat	HIV-1 NL4-3	shRNA (3 wk)	4 wk	細胞の生死

少）する蛋白質が多数報告されているがその詳細は各文献を照会されたい。報告されたこれらの蛋白質のHIV複製における役割の解明はこれからの課題である。

おわりに

以上，HIV-1複製を制御する宿主因子の探索について機能的ゲノムワイドsiRNA（shRNA）スクリーニングを中心に最近の知見を解説した。特にRNAi関連の実験技術やバイオインフォマティックスの進歩によって，HIV複製を制御する宿主因子に関する知見は日々蓄積しつつある。それぞれの方法論の改良や各方法の組み合わせによってHIV複製に関与する因子のさらなる発見とそれに基づいて開発される新たな作用機序を有する抗HIV剤の創出が期待される。

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SCIENCE STORY

HIVの粒子形成のメカニズム
―Gag蛋白に関する最新の知見―

HIVの*gag*遺伝子は、ウイルス粒子を構成する主要構造蛋白Gag蛋白をコードしている。Gag蛋白は感染後期過程において細胞質で合成され、形質膜への輸送、多量体化を経てゲノムRNAやEnv蛋白などを取り込んでウイルス粒子を形成し、出芽・放出される。この過程にはウイルス側の因子だけでなく、さまざまな宿主因子も関与していることが明らかになってきた。分子メカニズムの解明が進むに従い、この過程を標的とした新しい抗HIV薬の可能性もみえてきている。

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HIVの構造蛋白をコードする*gag*遺伝子

HIVの粒子は、図1のような構造をしています。大きくいえば、①エンベロープ（Env）蛋白が貫通した脂質二重膜とそれを裏打ちするようにマトリックス（MA）蛋白があり、そのなかに②キャプシド（CA）蛋白に囲まれて、③2本のゲノムRNAがヌクレオカプシド（NC）蛋白とともに存在する。さらに、逆転写酵素、インテグラーゼ、プロテアーゼといった酵素蛋白などがあるという構造です。

HIVのゲノムには、*gag*、*pol*、*env*という3つの主要遺伝子がありますが、MA、CA、NCといった、脂質二重膜より内側にあってウイルスの“殻”となる部分とウイルスの中心部分にある“芯”を形づくる構造蛋白をコードしているのが*gag*遺伝子なのです。

Gag蛋白前駆体Pr55^{Gag}

宿主細胞に吸着、侵入したHIVは、逆転写、インテグレーションを経て、宿主DNAに組み込まれます（感染前期過程）。この状態をプロウイルスと言いますが、そのDNAからウイルスのゲノムRNAと蛋白がつくられ、出芽、放出を経て、ウイルス粒子ができるわけです（感染後期過程）。この後期過程で、Gag蛋白が細胞内をどのように動き、ウイルス粒子が形成されるか、その分子メカニズムが明らかになってきました。

いまGag蛋白と言いましたが、これはプロウイルスの*gag*遺伝子から合成され、最終的にはHIV（HIV-1）の構造蛋白であるMA、CA、NCになる蛋白のことです。この蛋白は*gag*遺伝子から合成された直後は、MA、CA、NC、さらにp6などがつながった前駆体の形（Pr55^{Gag}）をしています（図2）。

宿主因子を利用したGag蛋白の輸送

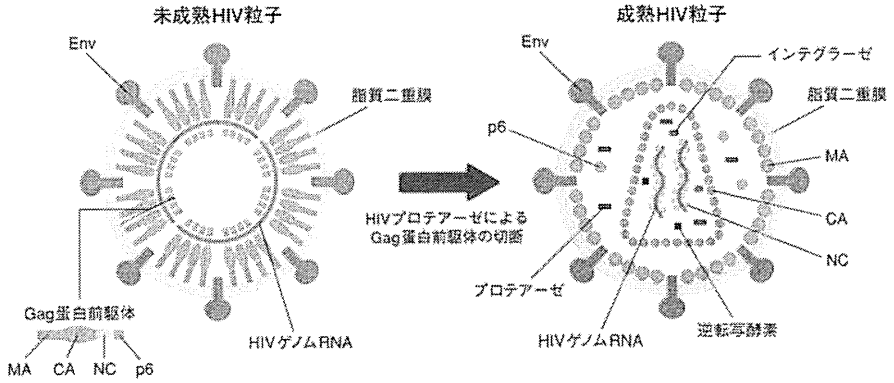
宿主細胞の細胞質で合成されたGag蛋白前駆体（以下、Gag蛋白）は、ウイルス粒子が形成される場へ輸送されていきます。これには細胞膜（形質膜）に直接、輸送され、粒子となって放出されるという説とMVB（multivesicular bodies）という細胞内小器官に輸送され、このMVBの中でウイルス粒子となったのちに形質膜から細胞外に放出されるという説があります（図3）。

HIVが感染するTリンパ球では形質膜直下、マクロファージではMVBでウイルス粒子が形成されるとも言われているものの、今のところ形質膜直下とする説が有力で、ここでもその説を中心に解説したいと思います。

最近になって、Gag蛋白の形質膜直下への輸送（trafficking、targeting）には、さまざまな宿主因子が使われることがわかってきました。宿主蛋白の細胞内輸送に関係すると考えられているAP-1、AP-2、AP-3、KIF4、Arf、GGA、そしてHIVの感染に伴って発現が上昇するSOCS-boxなどで、HIVはこうした宿主因子を拝借して

図1 HIV粒子の構造の模式図

宿主細胞からの出芽・放出時点では、マトリックス（MA）蛋白、キャプシド（CA）蛋白、ヌクレオカプシド（NC）蛋白はまだ形成されておらずGag蛋白前駆体の状態にある（未成熟HIV粒子：現時点の知見をもってしても未成熟HIV粒子の構造を図解することは困難であるので、ここでは極めて単純化して模式的に示す）。未成熟HIV粒子には感染性がなく、HIVのプロテアーゼにより未成熟HIV粒子内のGag蛋白前駆体がMA、CA、NCとなって、成熟HIV粒子が形成され、感染性を獲得する。



Gag蛋白を形質膜へ輸送していると考えられます。

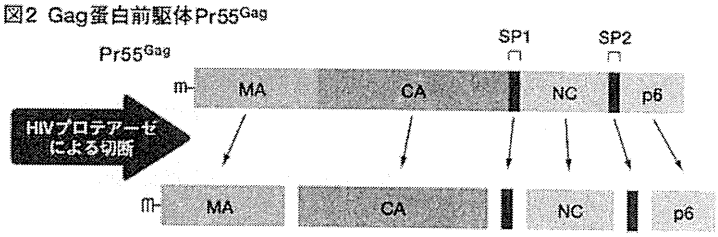
Gag蛋白の多量体化（アセンブリー）

1個のウイルス粒子形成には、数千ものGag蛋白が必要で、形質膜へ輸送されてきたGag蛋白は次にGag蛋白同士が連結する"多量体化（multimerization）"を起こします。そこにもウイルス側の因子だけでなく、宿主因子が関係しています。

宿主細胞の形質膜にあるホスファチジルイノシトール-(4,5)-2リン酸、PI(4,5)P_2というリン脂質がそれです。形質膜へ輸送されてきたGag蛋白は、MAの部分でこのリン脂質と結合します。すると、MA内に埋没していたミリストイル基が露出し、形質膜と強く結合するようになります（図4上段）。一方、Gag蛋白同士の結合にはCAの部分が重要と言われており、3量体程度になるとやはりミリストイル基が露出して、形質膜に結合できるようになります（図4下段）。ミリストイルスイッチモデルとして提唱されていて、これらはどちらもとGag蛋白の多量体化の引き金になるものと考えられています。

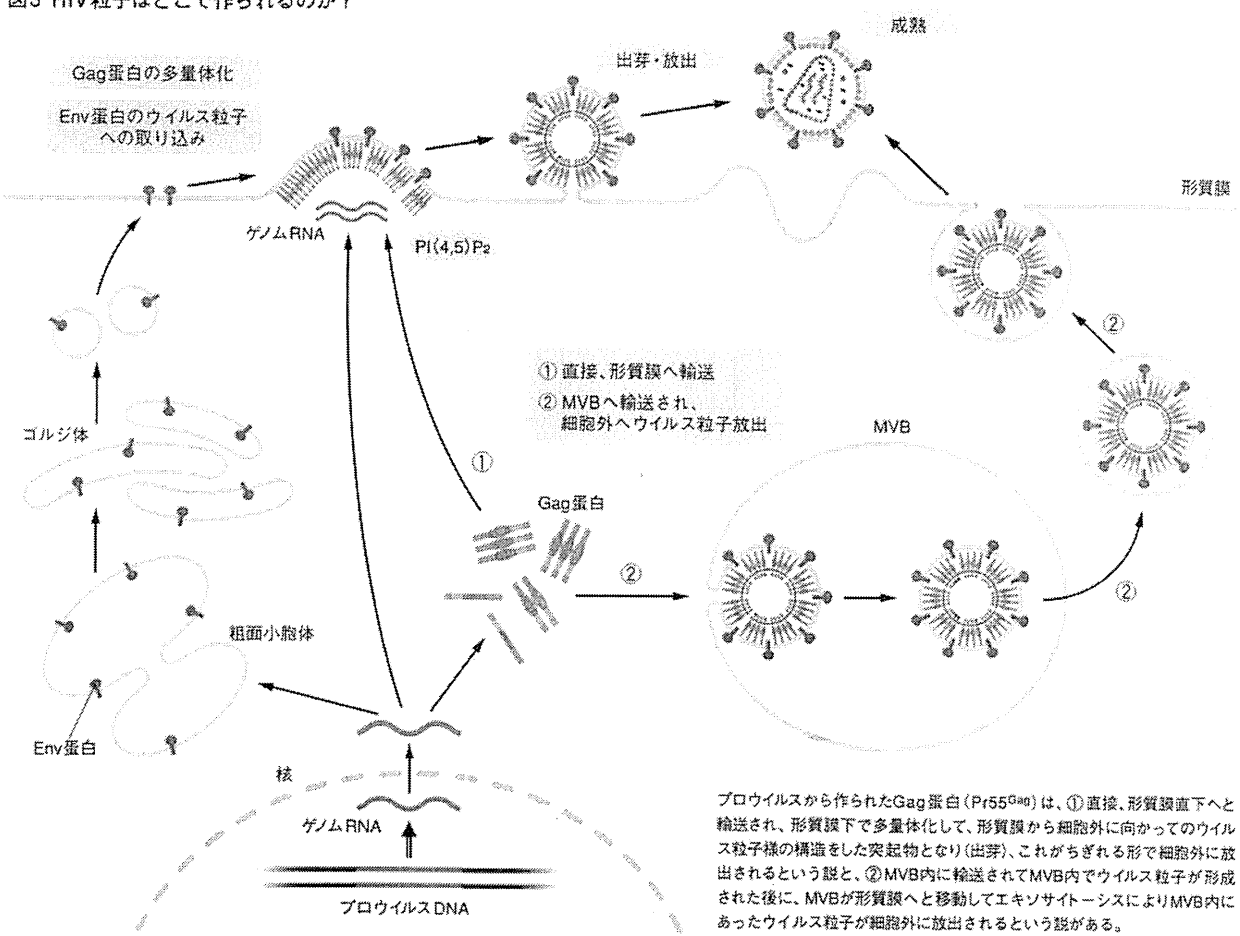
このほか、宿主のATP結合蛋白の1つであるABCE1が多量体の安定化に関係す

図2 Gag蛋白前駆体$Pr55^{Gag}$



Gag蛋白前駆体Pr55Gagはマトリックス（MA）、キャプシド（CA）、ヌクレオキャプシド（NC）とp6ドメインの4つの主領域と、CA-NC間、NC-p6間にあるSP1、SP2からなる。

図3 HIV粒子はどこで作られるのか？



プロウイルスから作られたGag蛋白（$Pr55^{Gag}$）は、①直接、形質膜直下へと輸送され、形質膜下で多量体化して、形質膜から細胞外に向かってのウイルス粒子様の構造をした突起物となり（出芽）、これがちぎれる形で細胞外に放出されるという説と、②MVB内に輸送されてMVB内でウイルス粒子が形成された後に、MVBが形質膜へと移動してエキソサイトーシスによりMVB内にあったウイルス粒子が細胞外に放出されるという説がある。

ることもわかってきています。

なお、Gag蛋白の多量体化のことを、狭い意味でアセンブリー（粒子形成、assembly）ということもあります。広い意味でアセンブリーといった場合は、Gag蛋白の輸送、多量体化、出芽、放出までのウイルス粒子形成過程全体を指します。

多量体化は、形質膜上でも特にコレステロールを多く含む"ラフト"と呼ばれる部位で起きているのではないかとも指摘されています。

出芽と放出にも宿主因子を利用する

多量体化したGag蛋白は、粗面小胞体→ゴルジ体→形質膜という経路で輸送されてきたEnv蛋白、さらにゲノムRNAを取り込んで、ウイルス粒子を形成し形質膜から出芽、放出されます（図3）。ゲノムRNAの取り込みには、Gag蛋白のなかのNCの部分が関係しているとされています。なお、ウイルス粒子の脂質二重膜は、宿主細胞の膜をそのまま拝借しています。

この出芽（budding）、放出（release）という過程にも、HIVは宿主が本来備えている機構を巧妙に利用していることがわかっています。現在、感染後期過程の分子メカニズムのうち、専門家のあいだで最もコンセンサスが得られているのがこの部分だと思います。

使われているのは、ESCRT（endosomal sorting complex required for transport、"エスコート"）という宿主因子複合体です。ESCRT Ⅰ、Ⅱ、Ⅲなどが知られていますが、宿主細胞は本来、不要となった受容体などをMVBに放出して捨てるためにこれらの蛋白を使っています。HIVのGag蛋白のp6の部分はESCRT Ⅰと相互作用し、ESCRT Ⅲを使って、ウイルス粒子を細胞外へ出していきます（図5）。このESCRT機構を阻害すると、ウイルス粒子は出芽するものの放出されなくなるため、特に出芽した粒子を"引きちぎる"ところに関係するものと考えられています。

放出されるウイルス粒子のなかでは、ウイルス自身がもっているプロテアーゼによってGag蛋白が順序よく切断され、成熟した感染性ウイルス粒子が組み立てられます。

新しい抗HIV薬の可能性は？

さて、HIV感染後期過程のこうした分子メカニズムが明らかになると、それらを標的とした新しい抗HIV薬の可能性が出てきます。

現在までに検討されているものとしては、Gag蛋白の多量体化に関係するCAを標的としたペプチドがあります。このペプチドはCAに結合してGag蛋白同士の相互作用を阻害するもので、*in vitro*では多量体化の阻害が認められています。別のペプチドでは感染性を抑制したとの報告もあります。

Gag蛋白のMAの部分のミリストイル化を抑えると、ESCRTを阻害したときと同じように、ウイルス粒子が出芽しかかりはするものの放出されないことも観察されています。

もちろん、Gag蛋白のp6の部分とESCRTなどとの相互作用を特異的に阻害できれば、有望な放出阻害薬となるはずです。

ウイルス粒子の成熟を阻害するbevirimatという薬剤は、現在、第Ⅱ相臨床試験に入っています。Gag蛋白のCAとSP1の間の切断を阻害するものですが、HIVプロテアーゼ阻害薬とは異なり、この部位へのプロアテーゼのアクセスそのものを阻害する作用をもっています。

宿主因子の立体構造やその作用分子メカニズムがわかれば、阻害薬をつくることは理論的には可能です。しかしながら、これまで概説しましたように後期過程にはさまざまな宿主因子が関係しており、効果と副作用の兼ね合いが難しいところがあると思います。宿主側に作用する薬では強い副作用が心配され、ウイルス側に作用する薬では耐性ウイルスが容易に選択される可能性があります。しかし、後期過程は抗HIV薬の新しい作用標的として期待が高く、今後の研究が待たれるところだと考えています。

図4 Gag蛋白の多量体化と形質膜への結合

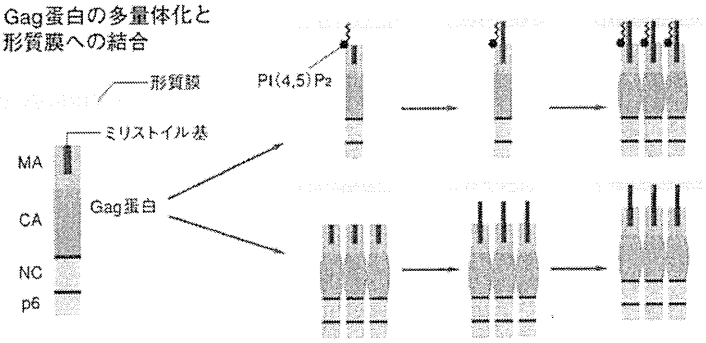
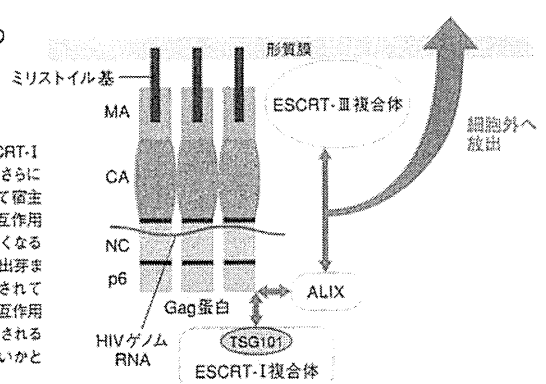


図5 p6とESCRT複合体の相互作用



Gag蛋白のp6部分が宿主のESCRT-Ⅰ複合体のTSG101の部分と結合、さらに宿主のALIXと結合し、これを介して宿主形質膜上にあるESCRT-Ⅲと相互作用する。これらの相互作用が働かなくなるような実験を行うと、HIV粒子は出芽までの状態にとどまることが観察されている。よって、これらの分子間の相互作用が宿主細胞からHIV粒子が放出される際の鍵になるメカニズムではないかと考えられている。

REVIEW
Future Microbiology

Role of CXCR4 in HIV infection and its potential as a therapeutic target

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The chemokine receptors CCR5 and CXCR4 are the two major coreceptors for HIV entry. Numerous efforts have been made to develop a new class of anti-HIV agents that target these coreceptors as an additional or alternative therapy to standard HAART. Among the CCR5 inhibitors developed so far, maraviroc is the first drug that has been approved by the US FDA for treating patients with R5 HIV-1. Although many CXCR4 inhibitors, some of which are highly active and orally bioavailable, have also been studied, they are still at preclinical stages or have been suspended during development. Importantly, the interaction between CXCR4 and its ligand SDF-1 is involved in various disease conditions, such as cancer cell metastasis, leukemia cell proliferation, rheumatoid arthritis and pulmonary fibrosis. Therefore, CXCR4 inhibitors have potential as novel therapeutics for the treatment of these diseases as well as HIV infection.

Approximately 34 million people are currently living with HIV, and 2 million people died due to AIDS or AIDS-related diseases in 2008 [201]. After the introduction of HAART in 1996, which combines HIV-1 reverse transcriptase and protease inhibitors, the morbidity and mortality associated with HIV-1 infection decreased dramatically [1,2] due to sustained reductions in HIV-1 plasma levels and significant increases in the number of $CD4^+$ T cells [3–5]. However, there are still several remaining problems associated with HAART that need to be overcome, such as the emergence of drug-resistant mutants and drug-related side effects. One approach to overcome these issues is to develop new reverse transcriptase or protease inhibitors that are effective against known drug-resistant mutations. Indeed, a new protease inhibitor, darunavir (TMC114), has been approved by the US FDA for the treatment of HIV/AIDS patients harboring multidrug-resistant HIV-1 variants that do not respond to existing HAART regimens [6]. In addition, etravirine (TMC125), a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI), has been recently approved by the FDA for the treatment of HIV infection in adults when other antiretroviral drugs have failed. Multiple mutations are required for HIV-1 to become resistant to etravirine in comparison to first-generation NNRTIs [7]. An alternative approach is to discover new anti-HIV drugs that are directed against a novel target and have a unique mechanism of action. In 2003, the FDA approved enfuvirtide, previously known as T-20, which targets HIV-1 gp41. Enfuvirtide is classified as a fusion inhibitor, which blocks fusion between the HIV-1 envelope and the target cell membrane. Although the drug must be administered parenterally, it is also effective against a multidrug-resistant HIV-1 strain [8,9]. In addition, the FDA approved the integrase inhibitor raltegravir in 2007 for use in highly treatment-experienced patients [10]. In addition, other anti-HIV-1 drug candidates have been explored as well, such as the maturation inhibitor bevirimat (PA-457) [11].

After the chemokine receptors CCR5 and CXCR4 were found to be the major HIV-1 coreceptors together with the primary receptor CD4, numerous efforts were made to test whether chemokines, chemokine derivatives, or small-molecule inhibitors against chemokine receptors had the potential to be a new class of anti-HIV-1 agent. In particular, many receptor antagonists against CCR5 have been developed as anti-HIV-1 drugs. Among them, maraviroc was approved by the FDA in 2007 for the treatment of R5 HIV-1 in treatment-experienced adult patients and is used in combination with other antiretroviral treatments. Several classes of CXCR4 antagonists have also been developed, although all have yet to reach clinical testing. However, other clinical applications for CXCR4 inhibitors in addition to anti-HIV therapy have been considered, as the interaction between CXCR4 and its ligand SDF-1 is also involved in several other diseases, such as

Keywords
▫ anti-HIV therapy ▫ cancer metastasis ▫ CCR5 ▫ chemokine receptor ▫ CXCR4 ▫ CXCR4 antagonist ▫ hematopoietic stem cell mobilizer ▫ HIV infection ▫ rheumatoid arthritis

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cancer metastasis, rheumatoid arthritis (RA) and pulmonary fibrosis [12]. In addition, recent research on bicyclam AMD3100, a well-known CXCR4 antagonist, has revealed that it specifically increases $CD34^+$ hematopoietic stem cell numbers in peripheral blood. A Phase III trial for AMD3100 as a stem cell mobilizer has been successfully completed [13]. In this review, we will highlight the role of CXCR4 in HIV infection and introduce the history and present status of various CXCR4 inhibitors. In addition, we will also describe the potential of CXCR4 as a therapeutic target in diseases other than HIV infection.

Identification of chemokine receptors as coreceptors for HIV-1 entry

Soon after the discovery that HIV-1 caused AIDS, CD4 was identified as the receptor for HIV-1 entry into target cells. However, it was recognized that a molecule was required in addition to CD4 to fully enable virus entry. One of the central observations that prompted this theory was the fact that human CD4 acts as a receptor only when expressed in human cells. It was then discovered that some chemokines, such as MIP-1α, MIP-1β and RANTES, inhibit HIV-1 infection, suggesting that the chemokines receptors may function as a coreceptor for HIV-1 entry [14]. Indeed, β-chemokine receptor CCR5, a member of the G protein-coupled receptor superfamily of seven-transmembrane domain proteins, was soon identified as the major coreceptor for M-tropic HIV-1 isolates, which can efficiently replicate in human macrophages and primary $CD4^+$ T cells [15–18]. Shortly after the discovery of CCR5, the α-chemokine receptor CXCR4 was reported as the main coreceptor for T-tropic HIV-1 isolates, which can efficiently replicate in T-cell lines and primary $CD4^+$ T cells [19]. Although the current consensus is that both CCR5 and CXCR4 are the major coreceptors for HIV-1 infection *in vivo*, additional chemokine receptors, including orphan receptors and others (e.g., CCR2b, CCR3, CCR8, CCR9, CXCR6 and CX_3CR1 [20]), have been reported to act as alternative coreceptors for CD4 when they are overexpressed.

The present model for HIV-1 fusion/entry is outlined in Figure 1 and is as follows:

- Binding of the gp120 trimer to CD4 induces conformational change(s) in gp120 that result in the exposure of high-affinity coreceptor binding sites;
- The interaction of gp120 with coreceptors then causes further conformational change(s) in gp120/gp41 that lead to exposure of the gp41 ectodomain;
- Extension of the gp41 fusion peptide causes a membrane fusion reaction between the viral membrane and the target cell membrane through formation of a six-helix bundle.

Determinants of HIV cell tropism & chemokine receptor usage

HIV-1 gp120 consists of five variable regions (V1–V5) and five constant regions (C1–C5). Cumulative evidence demonstrates that the V3 loop is a primary determinant of virus cell tropism [21–27] and coreceptor usage [16,28–31]. In addition, the V1/V2 region is also thought to regulate the efficiency of coreceptor-mediated HIV-1 entry [30,32]. Furthermore, conserved gp120/coreceptor binding sites have been proposed to exist mainly based on the fact that Env proteins from HIV-1, HIV-2 and SIV can interact with the same chemokine receptors (i.e., CXCR4 and CCR5).

Impact of nonfunctional chemokine receptor alleles on HIV resistance & disease progression

CCR5 δ32 is a mutant allele of CCR5 that is prevalent in European populations [33]. The gene product is nonfunctionally truncated such that the protein is unable to transport to the cell surface. Homozygotes for the δ32 allele exhibit a strong resistance to HIV infection, whereas heterozygotes show delayed progression to AIDS. Many other alleles are thought to affect the primary structure of CCR5 or its promoter, and some lead to nonfunctional receptors or otherwise influence AIDS progression [34,35]. For example, the CCR5 promoter polymorphism CCR5 59029G may affect viral transmission and disease progression due to its effect on the expression of CCR5. A single-nucleotide polymorphism (SNP) in the *CCR2b* gene has been identified as well. The presence of either one or two copies of this mutation, termed CCR-2b 64I, has been associated with delayed progression to AIDS and death in several cohorts [36]. Genome/polymorphism analysis of CXCR4 has so far failed to show a case equivalent to that of the δ32 allele. However, since SDF-1 interferes with X4-tropic HIV-1 infection, SDF-1 could be a genetic trait that might affect progression to AIDS. Indeed, a SNP in the 3′ untranslated region of SDF-1 was reported to be epidemiologically protective against HIV

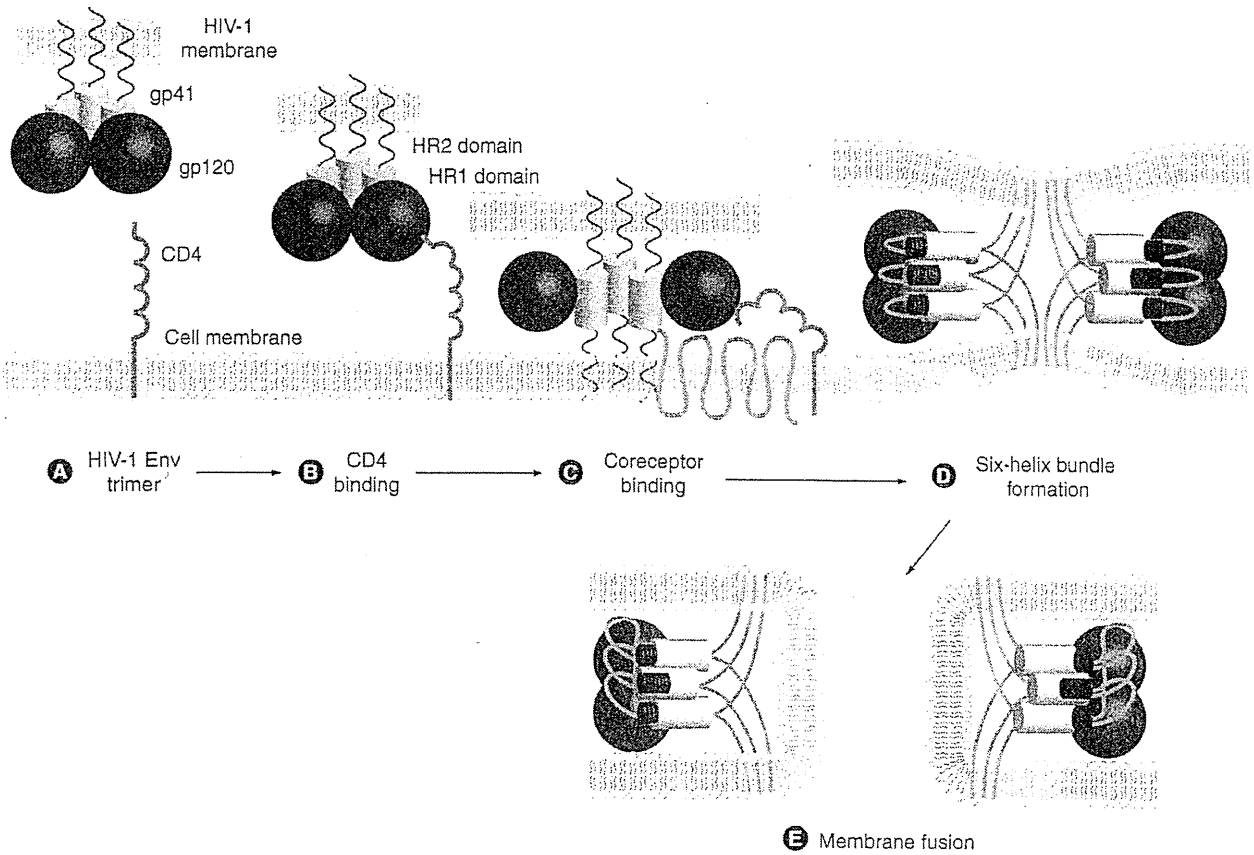

Figure 1. Proposed mechanism of HIV entry. The HIV Env protein is a homotrimeric protein that consists of surface gp120 and transmembrane gp41 proteins **(A)**. First, gp120 binds to the receptor CD4, and CD4 binding induces conformational changes in gp120 that lead to the exposure of a conserved gp120 domain and the gp41 ectodomain, which are important for coreceptor binding **(B)**. After binding to CD4, gp120 binds to a coreceptor, such as CXCR4 or CCR5, which is a member of the G-protein-coupled receptor superfamily of seven-transmembrane domain proteins **(C)**. Coreceptor binding can be blocked by many inhibitors that have been developed against CXCR4 and CCR5. Coreceptor binding causes further conformational changes in HIV Env that allow extension of the gp41 fusion peptide and generation of a six-bundle formation **(D)** following membrane fusion between the viral and target cell membrane **(E)**.

infection and progression [37]. In contrast to CCR5 δ32, this mutation is rather common in all geographical regions of the world. It has been suggested that this mutation may increase SDF-1 levels, thus competing with X4 HIV binding to cells. Those who are homozygous for this mutation demonstrate slow progression to AIDS without exhibiting decreased susceptibility to HIV infection [37]. Mutations in the *CXCR4* gene are generally rare and have not been implicated in HIV-1/AIDS pathogenesis.

Coreceptor-targeted anti-HIV therapy

Along with CCR5, CXCR4 is a major coreceptor in HIV infection. R5 HIV-1 is isolated predominantly during the acute and asymptomatic stages [38], whereas X4 HIV-1 strains emerge in approximately 50% of infected individuals, and their emergence is associated with rapid $CD4^+$ T cell loss and disease progression [39,40]. CXCR4, therefore, is a novel and attractive target for the development of new anti-HIV drugs. Significant efforts have been made to explore and identify small-molecule compounds that interfere with the interaction between gp120 and CXCR4. The structures of some of the well-studied CXCR4 inhibitors discussed below are shown in Table 1 & Figure 2.

AMD3100 & AMD070

Bicyclam AMD3100 (Table 1 & Figure 2) is a small molecule inhibitor that strongly restricts X4 HIV-1 infection [41]. The compound exhibits no antiviral activity against CCR5-utilizing HIV-1 strains. Correlation was observed between the inhibitory activity of AMD3100 on X4 HIV-1 replication, CXCR4 mAb binding and SDF-1α-induced signal transduction. Thus, AMD3100 is a specific antagonist of CXCR4. AMD3100 has proven effective not only in a severe combined

Table 1. CXCR4 and CCR5 inhibitors tested in HIV and other applications.

Compound	Company	Stage of development	Disease	Note
CXCR4 inhibitors				
ALX40-4C	NPS Allelix	Terminated (Phase I/II)	HIV	No apparent effect was observed on viral load
AMD3100	AnorMED	Terminated (Phase I/II)	HIV	Little effect was observed on viral load
AMD3100 (plerixafor)	Genzyme	Approved by US FDA	Stem cell mobilizer	Use in combination with G-CSF
AMD070	Genzyme	Suspended (Phase I/II)	HIV	A derivative of AMD3100 that can be orally administered. Liver histology changes were observed in long-term preclinical toxicity experiments.
T140	Kyoto University	Preclinical	HIV, cancer metastasis, leukemia, rheumatoid arthritis	A downsized analog of T22 peptide that specifically inhibits CXCR4
KRH-3955	Kureha	Preclinical	HIV, cancer metastasis	A highly potent, orally bioavailable CXCR4 antagonist
CCR5 inhibitors				
TAK-652 (TBR-652)	Takeda/Tobira	Phase II	HIV	A potent, orally bioavailable CCR5 antagonist
Aplaviroc	Ono	Terminated (Phase II/III)	HIV	Aplaviroc's development was stopped because of hepatotoxicity
Maraviroc	Pfizer	Approved by US FDA	HIV	The first FDA-approved CCR5 antagonist
PF-232798	Pfizer	Phase II	HIV	A second-generarion Pfizer oral CCR5 antagonist
Vicriviroc	Schering-Plough/Merck	Terminated (Phase III)	HIV	Vicriviroc did not meet the primary efficacy endpoint
INCB9471	Incyte	Phase II	HIV	A new class of oral CCR5 antagonist

G-CSF: Granulocyte colony-stimulating factor.

immunodeficiency (SCID)-hu/Thy/Liv mouse model [42] but also in a proof-of-concept study on a patient infected with X4 HIV-1. Development of the compound as an anti-HIV drug was suspended primarily due to its cardiotoxicity [43]. An effort to overcome the poor oral bioavailability and side effects of AMD3100 led to the generation of AMD070 (Table 1 & Figure 2), which has tetrahydroquinolineamine as its pharmacophore and which is a potent and specific X4 HIV-1 inhibitor with high oral bioavailability. Although AMD070 also showed potential activity against X4 HIV-1 in a clinical Phase Ib/IIa proof-of-concept study, the FDA halted development of this compound due to liver histology changes in long-term preclinical toxicity studies [44]. Further studies are needed to determine *in vivo* toxicity of AMD3100 and AMD070, is due to blocking CXCR4 functions.

T22, T134 & T140

Self-defense peptides isolated from the hemocytes of the Japanese and American horseshoe crab tachyplesins and polyphemusins have antibacterial and antiviral activities [45–47]. Several years prior to the discovery of the HIV-1 coreceptors, Yamamoto and colleagues were able to show that T22 ([Tyr5, 12, Lys7]-polyphemusin) (Table 1 & Figure 2), a synthetic polyphemusin analog with 18 amino acid residues and two disulfide bonds, inhibits HIV-1 replication [47,48]. The two disulfide bonds form an antiparallel β-sheet structure, which is important for the antiviral activity of the peptide. The peptide inhibits replication of T-tropic but not M-tropic HIV-1. The determinant of this specific antiviral activity was mapped to the V3 region of the HIV-1 Env protein [49], and the 50% effective concentration (EC_{50}) was 290 nM in an anti-HIV assay using MT-4 cells. Subsequent to the discovery of the CXCR4 and CCR5 coreceptors, we demonstrated that the T22 peptide specifically blocks virus–cell and cell–cell infection mediated through HIV-1 Env interaction with CXCR4 and CD4 [50], as also reported in other CXCR4 inhibitors such as ALX40-4C and AMD3100 [41,51]. It was also found that T22 suppresses Ca^{2+} mobilization induced by SDF-1α. Thus, T22 is a small CXCR4 antagonist that inhibits X4 HIV-1 infection via specific binding to the CXCR4 molecule.

Through an effort to downsize T22 by structure–activity relationship studies, it was found that T134 [52] and T140 [53] (Table 1 & Figure 2),

14-mer peptides with a single disulfide bond, had stronger anti-HIV-1 activity than T22. Although T140 lacks four amino acids and one disulfide bond, it maintains an antiparallel β-sheet structure. Through an Ala-substitution scanning study, it was demonstrated that Arg2, Nal3, Tyr5 and Arg14 form the pharmacophore of T140, which is useful information for the rational design of peptide derivatives with higher anti-HIV activity [54]. Downsizing T140 resulted in the development of a cyclic pentapeptide, FC131, which has strong CXCR4-antagonistic activity and could serve as a template for further modification [55]. Systemic toxicity of the T compounds was evaluated with TN14003, a derivative of T140 [56]. CB-17 SCID mice were injected with TN14003 at 100 ng/g body weight twice weekly for 45 days. Although no damage in the liver and kidney was observed by hematoxylin and eosin staining, further careful *in vivo* study is required for assessing the safety of the T compounds.

KRH-1636 & KRH-3955

In an attempt to discover a novel, small non-peptide CXCR4 antagonist Yamamoto and colleagues screened more than 1000 compounds from the Kureha Corporation (Tokyo, Japan) chemical library. This screen led to the identification of KRH-1636 (Table 1 & Figure 2), which strongly and specifically inhibits X4 HIV-1 replication, both *in vitro* and *in vivo* [57]. KRH-1636 blocks the replication of various X4 HIV-1 strains in a nanomolar range and has low cytotoxicity (CC_{50}: 400 μM in MT-4 cells). KRH-1636 also strongly inhibits both SDF-1α binding to CXCR4 and CXCR4-mediated Ca^{2+} signaling and blocks binding of monoclonal antibodies to CXCR4 without down-modulating the coreceptor. Importantly, KRH-1636 also inhibits X4 HIV-1 replication in a human peripheral blood lymphocyte (PBL)/ SCID mouse model. Furthermore, KRH-1636 was absorbed into the blood after intraduodenal administration in rats. KRH-1636 did not show severe toxicity in rats that received the compound (1.5 mg/kg per day) by intravenous administration for 4 days [57].

Vigorous efforts to search for more potent and orally bioavailable CXCR4 antagonists were undertaken through a combination of chemical modification of KRH-1636 and biological assays, leading to the identification of KRH-3955 (Table 1 & Figure 2) [58]. KRH-3955 very potently inhibits replication of X4 HIV-1 in activated peripheral blood mononuclear cells (PBMCs) from different

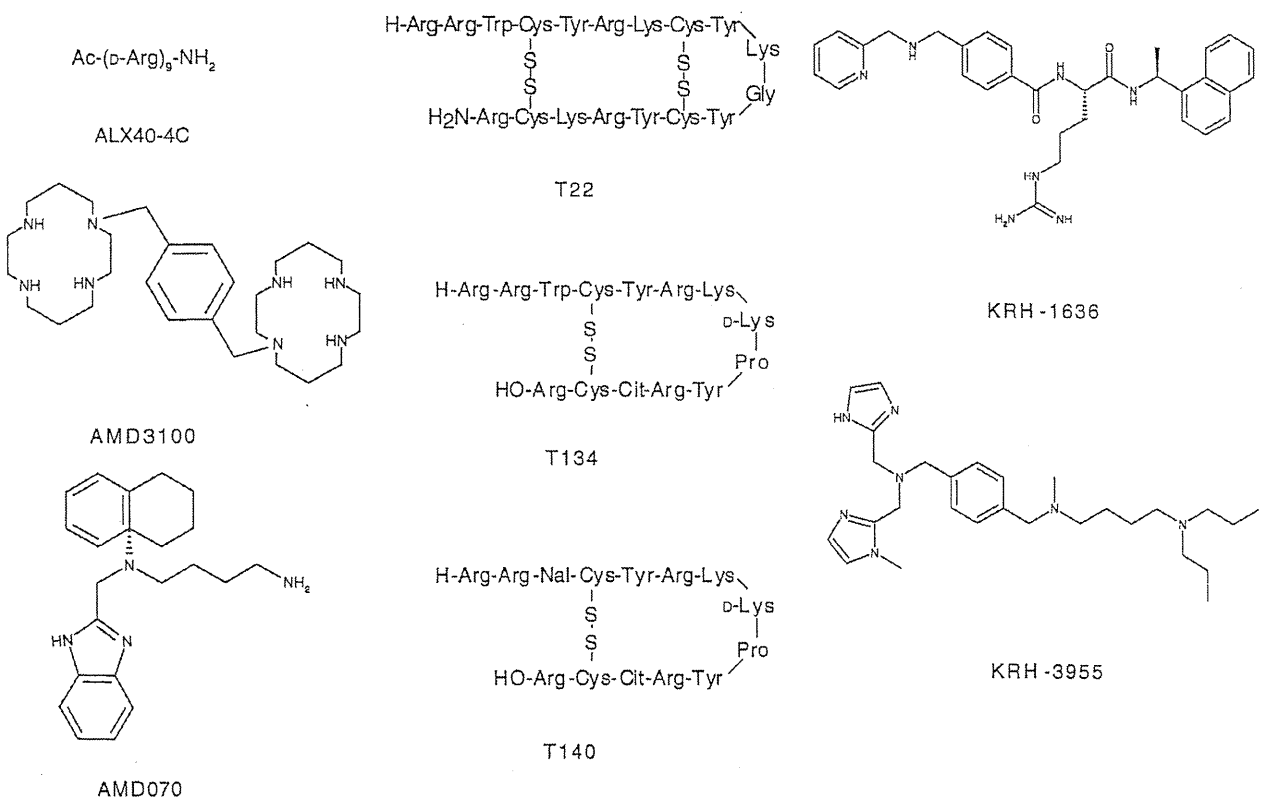

Figure 2. CXCR4 inhibitors.

donors and effectively restricts clinical HIV-1 isolates. The EC_{50} of KRH-3955 is approximately 1 nM, and it also blocks replication of recombinant X4 HIV-1 containing resistance mutations in reverse transcriptase and protease, as well as isolates with T-20-resistant mutations in the envelope protein. KRH-3955 inhibits both SDF-1α binding to CXCR4 and Ca^{2+} signaling through the coreceptor. Moreover, KRH-3955 does not induce CXCR4 internalization but inhibits the binding of anti-CXCR4 monoclonal antibodies that recognize the second or third extracellular loops of the receptor. The compound shows an oral bioavailability of 26% in rats and oral administration blocks X4 HIV-1 replication in the human PBL/SCID model. Thus, KRH-3955 is a strong CXCR4 antagonist and seems to be a new promising therapeutic agent against HIV-1 infection and AIDS although further studies are definitely needed on *in vivo* safety of the compound.

Other CXCR4 inhibitors

In addition to the compounds above, there are several other CXCR4 inhibitors. These include: ALX40-4C (Table 1 & Figure 2) [51,59] and Arg-mimetic conjugates [60,61], POL3026 (β-hairpin mimetic) [62,63] and CGP64222 [64]. ALX40–4C, a polypeptide of nine D-Arg residues stabilized by terminal protection, inhibits X4 HIV-1 infection as well as R5X4 (dual-tropic) HIV-1 infection in the context of CXCR4 use [51]. The peptide also blocks binding of SDF-1α and the anti-CXCR4 monoclonal antibody 12G5 to CXCR4. Although no significant reduction in viral load was observed, ALX40–4C was well tolerated by 39 out of 40 HIV-infected individuals for a 1-month treatment period in Phase I/II clinical trials [59]. The β-hairpin motif in the polyphemusin and T22, which are described in the previous section, was used to design a β-hairpin mimetic POL3026. The cyclic peptide specifically inhibits X4 HIV-1 infection [62]. Importantly, POL3026 showed excellent stability in human plasma and favorable pharmacokinetics when administered subcutaneously in dogs [63].

Molecular interactions between CXCR4 inhibitors & the CXCR4 receptor

The molecular interactions between CXCR4 inhibitors or antagonists with the CXCR4 receptor have been examined primarily using three methods. The first method involves examining the effect of CXCR4 inhibitors on the binding of several anti-CXCR4 monoclonal antibodies that recognize various regions of the receptor. In the second, the effect of mutations in CXCR4 on the inhibitory activity of CXCR4 antagonists is investigated together with binding of certain anti-CXCR4 monoclonal antibodies, such as 12G5, or the ligand SDF-1α to the receptor. Thirdly, radio or photo-labeled inhibitors are used to identify the binding sites of inhibitors directly.

Using various CXCR4 mutants, the determinants of AMD3100 sensitivity were identified as four Asp residues at positions 171, 182, 193 and 262, as well as Glu288, indicating the importance of the second extracellular loop (ECL)2 and ECL3 and their connected transmembrane domains (Figure 3) [65–68]. Reduced binding of radiolabeled T140 was observed when CXCR4 was mutated at Asp171, Arg188, Tyr190, Gly207 and Asp262, again suggesting the contribution of ECL2 to inhibitor binding [69]. Furthermore, T140 photolabeling recently revealed that the peptide actually interacts with the fourth transmembrane domain of CXCR4 [70]. To determine the binding site(s) of KRH-3955, its effect on the binding of four different anti-CXCR4 monoclonal antibodies was examined, and the data obtained suggested that KRH-3955 binds a region composed of all three CXCR4 ECLs. Further studies were performed using various CXCR4 point mutants to narrow down the binding site(s) of KRH-3955. While AMD3100 lost its activity when Asp171, Asp262 or Glu288/Leu290 was mutated, unexpectedly, His281 was the only residue whose mutation affected the inhibitory activity of KRH-3955 (Figure 2) [58]. Together, these data indicate that several acidic amino acid residues in CXCR4, such as Asp171 and Asp262, are crucial for the binding of most of the CXCR4 inhibitors.

CCR5-targeted anti-HIV therapy

Initially, natural CCR5 ligands, such as MIP-1α and RANTES, or their protein (or peptide)-based inhibitors, were studied as potential CCR5 inhibitors. However, these molecules were not developed further as HIV therapeutics due to their poor pharmacokinetics and bioavailability. Several small molecule CCR5 antagonists have been reported to date (Table 1): TAK-779 [71], TAK-652 (TBR-652) [72] and TAK-220 [73] (Takeda; Osaka, Japan), AK602 (presently termed aplaviroc; Ono; Osaka, Japan) [74], SCH-C [75] and SCH-D (renamed vicriviroc) [76] (Schering-Plough/Merck), UK-427,857 (presently termed maraviroc) [77], PF-232798 [78] (Pfizer; Sandwich, UK) and INCB9471 (Incyte, DE, USA) [79]. Among them, the FDA approved maraviroc in 2007, for

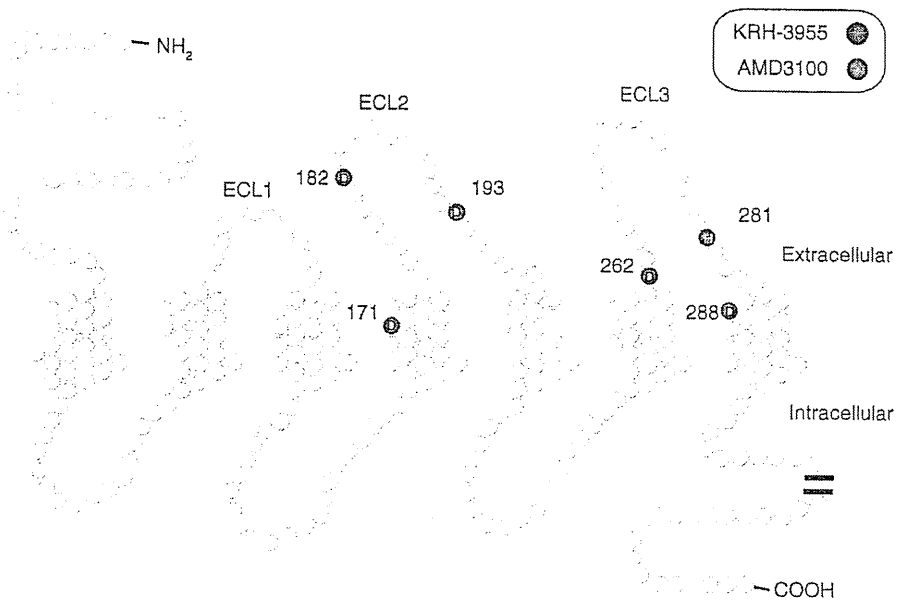

Figure 3. Serpentine diagram of the CXCR4 receptor. White letters in blue circles and black letters in red circles represent amino acid residue substitutions that severely reduce the inhibitory activities of AMD3100 and KRH-3955, respectively.
ECL: Extracellular loop.

treatment-experienced adult patients, in combination with other antiretroviral drugs. Vicriviroc is being terminated in Phase III clinical trials.

Resistance to coreceptor inhibitors

Theoretically, HIV-1 acquires resistance to coreceptor inhibitors, including coreceptor ligands or anti-coreceptor antibodies, either by changing the way it uses coreceptors or switching coreceptor usage, such as shifting from CCR5 to CXCR4. Experiments to select viruses resistant to coreceptor inhibitors have been mainly performed in two ways. In one approach, a selection experiment is performed using cell lines that express only CXCR4 or CCR5 [80–84]. In this case, resistant viruses did not show coreceptor switching, but rather started to use the same coreceptor in a drug-bound form. Many mutations in resistant HIV-1 were found in the gp120 region of HIV-1 Env, especially in the V2 and V3 regions. It is generally considered that it is much more difficult for the virus to gain resistance to coreceptor inhibitors than to HIV-1 inhibitors that target viral proteins such as reverse transcriptase. Indeed, 145 passages over 1.5 years were required to obtain T134-resistant NL4–3, which shows modest resistance to the T134 peptide (15-fold increase) [84]. The T134-resistant HIV-1 is also resistant to AMD3100 as well (15-fold increase). Interestingly, effective concentration of T134 against AMD3100-resistant HIV-1 is almost the same as that against the wild-type strain. In the second resistant virus generation strategy, selection experiments are performed using a cell that expresses both CXCR4 and CCR5, such as human PBMC or PBL [85–95]. Sequence analysis of resistant variants revealed different patterns of amino acid changes in the envelope regions. In some cases, resistant mutations were mainly located in the V3 region of the HIV-1 envelope. Amino acid changes also occurred throughout gp160 in the absence of changes in the V3 loop [89]. Furthermore, detailed analysis revealed that three physically proximal mutations in the gp41 fusion peptide region are responsible for viral resistance to vicriviroc [93]. It is of note that most HIV-1 variants with resistance to CCR5 inhibitors remain CCR5-tropic, although emergence of CXCR4-using variants from SF162 was also reported in cell culture passage in the presence of maraviroc [94].

Another important issue in viral drug resistance is whether resistance mutations affect virus fitness and/or sensitivity to neutralizing antibodies. Reduced fitness was reported for HIV-1 isolates resistant to CXCR4 inhibitors such as AMD3100 [96]. On the other hand, resistance to the CCR5 inhibitor AD101 (SCH-30851, a precursor of vicriviroc) is not associated with a fitness loss [87]. Escape mutants from two

different CCR5 inhibitors, AD101 and vicriviroc, were examined for their sensitivity to well-known neutralizing monoclonal antibodies, such as b12, 2G12, 2F5 and 4E10, as well as to sera from HIV-1-infected individuals [92]. The rationale for this study is that at least some of the escape mutants against CCR5 inhibitors change amino acids in their envelope region(s), which can be targets for neutralizing antibodies. Interestingly, the escape mutants were more sensitive than the parental isolates to a subset of the neutralizing antibodies and some sera from HIV-1-infected individuals, indicating that the humoral immune response could exert selection pressure *in vivo*. Further studies will be required to more completely examine how drug resistance alters virus fitness and/or susceptibility to the immune system.

Several studies have been reported on clinical resistance to CCR5 inhibitors. Coreceptor tropism was examined of 64 HIV-1-infected patients who were given short-term monotherapy of maraviroc. Phylogenic analysis of CXCR4-using variants selected only in two patients revealed that those variants were most likely derived from a CXCR4-using reservoir [97]. Clinical resistance to vicriviroc was examined using both subtype C and subtype D of HIV-1. In the case of an HIV-1 subtype C-infected subject, amino acid changes within the V3 loop were sufficient to confer vicriviroc resistance [98]. On the other hand, amino acid changes in the V3 loop as well as the C4 domain are fully responsible for vicriviroc resistance in HIV-1 subtype D [99].

Other applications of CXCR4 inhibitors

Anticancer

CXCR4 is expressed constitutively in a wide variety of normal tissues, including lymphoid tissues, thymus, brain, spleen, stomach and the small intestine [100]. The SDF-1/CXCR4 interaction is critical for normal lymphocyte trafficking and homing and retention of hematopoietic stem cells within the bone marrow, and is essential in fetal hematopoiesis [101]. Interaction of SDF-1 with CXCR4 activates effector molecules that regulate cell survival, proliferation, chemotaxis, migration and adhesion. Growing evidence suggests that a number of chemokine receptors, most notably CXCR4, are also deeply involved in cancer pathogenesis, such as cancer growth, metastasis and angiogenesis [102]. Abundant CXCR4 expression is reported in tumor cells in more than 23 various types of human cancers of epithelial, mesenchymal and hematopoietic origin [103]. This includes tumors such as breast cancer, ovarian cancer, hepatocellular carcinoma and hematologic malignancies, as well as a number of other malignancies, including lung, brain and prostate cancer.

Cancer metastasis is the result of several sequential steps and represents a highly organized, nonrandom and organ-selective process [104]. Activation of CXCR4 not only results in cytoskeletal changes leading to cell migration, but also induces the production of matrix metalloproteinases at the primary tumor site [105]. This is important for detachment of the tumor cells and their migration through the extracellular matrix into the circulation. Cancers can also have diverse metastatic patterns involving the regional lymph nodes, bone marrow, lung and liver. Muller *et al.* were the first to show that CXCR4 is highly expressed in human breast cancer cells, malignant breast tumors and metastatic tumors, and that in mice CXCR4-expressing breast cancer cells aggressively metastasized to secondary organs where SDF-1 expression is significantly higher [106]. Blocking SDF-1/CXCR4 interaction with a neutralizing antibody significantly inhibited the metastasis of breast cancer cells to distant organs [106]. Other studies have shown that a CXCR4 antagonist peptide (CTCE-9908) reduces metastasis consistently in many different murine cancer models [107,108]. Similar *in vivo* observations have been made with CXCR4 blocking small molecules, such as AMD3100 (ovarian carcinoma) [109], peptides (melanoma) [110], TN14003 (head and neck cancer) [111], antibodies (prostate cancer) [112] and a siRNA (breast cancer) [113]. Indeed, AMD3100 (Genzyme Corporation; MA, USA) is currently being tested for hematologic malignancies in a Phase I/II trial in combination with other treatments such as chemotherapy. To define the spontaneous metastasis of breast cancer cells, we also conducted histological examinations using various organs of NOD/SCID/γ(null) (NOG) mice inoculated with CXCR4-low-expressing MCF-7 and CXCR4-high-expressing MDA-231 cell lines [102]. Results from this study showed that NOG mice inoculated with CXCR4-low-expressing MCF-7 cells barely exhibited organ metastasis, whereas spontaneous metastasis of tumor cells was found in the lungs of NOG mice inoculated with CXCR4-high-expressing MDA-231 cells. These results suggest that CXCR4-high-expressing tumor cells could spontaneously metastasize to secondary organs.

We also examined the effect of KRH-1636 and its derivatives on SDF-1α-mediated chemotaxis of cancer cells to address whether these molecules could be applied to abrogate cancer metastasis [114]. We found that two derivatives, KRH-2731 and KRH-3955, potently antagonize SDF-1α-mediated chemotaxis, exhibiting an EC_{50} of less than 10 nM, which was more than a 1000-fold improvement in efficacy over the prototype KRH-1636. As far as we analyzed the KRH compounds tested, there was a correlation of inhibition among SDF-1α binding, SDF-1α-mediated chemotaxis and HIV-1 infection [58,114]. Therefore, the KRH-1636 derivatives KRH-2731 and KRH-3955 may be promising as novel anticancer metastasis drugs in addition to potential HIV-1 therapeutics.

Although less well studied compared with metastasis, the effects of CXCR4 on survival, proliferation, angiogenesis and vasculogenesis of cancer cells are also important. SDF-1 produced by stromal cells may induce a survival or antiapoptotic signal in tumors that reduces their susceptibility to current treatments, such as chemotherapy [108]. It has been reported that in ovarian, glioma, small-cell lung, renal and thyroid cancers, SDF-1 can stimulate the proliferation and/or survival of CXCR4-expressing cancer cells when they are grown under suboptimal conditions, such as low serum concentrations [102,115]. To define the role of SDF-1/CXCR4 interaction in breast cancer cell proliferation and apoptosis, we cultured the breast cancer cell line MDA-321 in serum-depleted media. MDA-321 cells depend on serum for maximal growth in culture. A total of 24 h after serum withdrawal, MDA-321 cells exhibited a significant decrease in cell number and underwent apoptosis. However, when serum was withdrawn in the presence of SDF-1, there was almost no decline of cell number and no apoptotic cells were observed. Two breast cancer cell lines (CXCR4-low-expressing MCF-7 and CXCR4-high-expressing MDA-231 cells) were inoculated subcutaneously in the postauricular region of NOG (NOD/SCID/gc null) mice. CXCR4-low-expressing MCF-7 cells formed a small tumor at the inoculation site in NOG mice after 8–9 weeks. By contrast, CXCR4-high-expressing MDA-231 cells produced a large tumor within 2–3 weeks in NOG mice [Dewan *et al.*, Unpublished Data]. These data suggest that SDF-1/CXCR4 interaction might play an important role in breast cancer cell proliferation and prevention of apoptosis and in primary tumor growth in SCID mice at the inoculation site. The effects of CXCR4 on cancer growth can be modulated, as inhibition of CXCR4 with the CXCR4 antagonist AMD3100 reduced proliferation and increased apoptosis of human brain cancer cells [116]. In this study, systemic administration of AMD3100 inhibited the growth of intracranial glioblastoma and medulloblastoma xenografts and increased tumor cell apoptosis within 24 h [116]. Thus, CXCR4 signaling may promote cancer through a wide range of mechanisms, including proliferation and survival of cancer cells, angiogenesis and chemoinvasion of cells at primary and metastasis sites.

Antiarthritis & allergy

In addition to HIV infection, cancer cell metastasis and leukemia cell proliferation, the SDF-1–CXCR4 system has been implicated in several other pathological conditions, such as RA, allergy and pulmonary fibrosis. AMD3100 significantly improves the symptoms of these diseases [13,43], and the 14-mer peptide T140 and its analogs, initially characterized as potent anti-HIV inhibitors, were also found to be anti-RA agents [117].

Hematopoietic stem cell mobilization

Xiao *et al.* tested whether stem cell number and function were related to *CXCR4* gene variation. Stem cell number was inversely associated with SDF-1α levels, and an *SDF-1* gene variation (rs2297630) influenced SDF-1α levels and circulating stem cell number. Moreover, the plasma SDF-1α level is a predictor of stem cell number [118]. Transplantation of stem cells harvested from peripheral blood is an important treatment option for hematological malignancies. Though the cytokine granulocyte colony-stimulating factor (G-CSF) has frequently been used to mobilize stem cells, it is not sufficient alone for effective autologous transplantation. Preclinical studies have demonstrated that AMD3100 (now known clinically as plerixafor; Genzyme Corporation) can rapidly and reversibly mobilize hematopoietic stem cells into peripheral circulation in synergy with G-CSF [13,119]. These data suggest that plerixafor could potentially be useful in treating hematological disease. At the end of 2008, the FDA approved a plerixafor solution for subcutaneous injection (Mozobil™) for use in combination with G-CSF to mobilize hematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients with non-Hodgkin lymphoma and multiple myeloma.

Conclusion

The discovery of HIV coreceptors, such as CCR5 and CXCR4, has prompted a search for inhibitors against these receptors as an alternative therapy

to HAART. Although new anti-HIV drugs that target HIV gp41 and integrase have recently been approved, inhibitors against CCR5 and CXCR4 represent the first anti-HIV agents that target host proteins rather than viral enzymes or proteins. The FDA approved the CCR5 inhibitor maraviroc in 2007 in combination with other antiretroviral drugs for use in treatment-experienced adult patients with R5 HIV-1. Several types of CXCR4 inhibitors have also been developed, such as the bicyclam AMD3100, peptide T140 and nonpeptide small molecules such as KRH-3955, and these molecules have been extensively studied. Some of these CXCR4 inhibitors are not only highly active against HIV but are also orally bioavailable; however, they are still in preclinical stages or have been suspended during development because of unforeseen side effects. The interaction between CXCR4 and its ligand SDF-1 also plays important roles in the migration of progenitor cells during embryonic development of various systems, such as the hematopoietic system. Indeed, AMD3100 has been shown to induce the rapid and reversible mobilization of hematopoietic stem cells into peripheral circulation in synergy with G-CSF. Furthermore, the SDF-1–CXCR4 axis has been found to play critical roles in various disease conditions, such as cancer cell metastasis and growth, RA, allergy and pulmonary fibrosis. These results indicate that CXCR4 inhibitors could be used to treat not only HIV infection, but also various diseases associated with CXCR4.

Future perspective

There are at least two important issues that will need to be addressed when coreceptor inhibitors are used as clinical drugs. First, it is necessary to determine which combination of entry inhibitors should be used once CXCR4 inhibitors are available for clinical use. In this scenario, it is reasonable to use CCR5 inhibitors, such as maraviroc, in combination with CXCR4 inhibitors because:

- It is not neccessary to assess the amount of R5, R5X4 and X4 HIV in the patients before initiating treatment with coreceptor inhibitors;
- Combination of a CCR5 inhibitor with CXCR4 inhibitors shows more potent synergism compared with the synergy obtained with other anti-HIV drugs, although the precise mechanism for this synergy will need to be clarified [120].

In addition, this combination could limit evolution and switching of coreceptor usage in both R5 and X4 HIV strains. Alternatively, coreceptor inhibitors could be used with fusion inhibitors such as enfuvirtide. In fact, synergistic inhibition has been reported when enfuvirtide and CCR5 inhibitors are combined [121,122].

The other important issue is the long-term safety of CXCR4 inhibitors. As mentioned above, the interaction between CXCR4 and its ligand SDF-1 plays important roles in the migration of progenitor cells during embryonic development of the cardiovascular, central nervous and hematopoietic systems. In addition, the SDF-1–CXCR4 axis has been found to be involved in various disease conditions such as cancer cell metastasis and RA. At the same time, however, inhibiting SDF-1α–CXCR4 interactions may evoke severe adverse effects. It has been reported that knocking out either the *SDF-1α* or the *CXCR4* gene in mice causes marked defects, such as abnormal hematopoiesis and cardiogenesis in addition to vascularization of the gastrointestinal tract [123–125]. Thus, an important point to be considered is whether blocking CXCR4 functions can affect animals at postdevelopmental stages, although approval of plerixafor (AMD3100) as a stem cell mobilizer indicates that at least short-term blockade of SDF-1/CXCR4 is safe in humans. Further careful studies for long-term use of CXCR4 inhibitors will certainly be required to properly address this question.

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Executive summary

Identification of chemokine receptors as coreceptors for HIV-1 entry

- CXCR4 and CCR5 are the two major coreceptors for HIV-1 infection.
- Coreceptors are responsible for a crucial step between Env binding to the receptor CD4 and membrane fusion mediated by the gp41 fusion peptide.

Determinants of HIV cell tropism & chemokine receptor usage

- The V3 loop of HIV-1 gp120 is a primary determinant of cell tropism and chemokine receptor usage, whereas the V1/V2 region is involved in effective entry of the virus.

Impact of nonfunctional chemokine receptor alleles on HIV resistance & disease progression

- Homozygotes and heterozygotes for CCR5 δ32, which are prevalent in European populations, exhibit a strong resistance to HIV infection and delayed progression to AIDS, respectively.
- A single-nucleotide polymorphism in CCR2b, one of the minor coreceptors for HIV, is associated with delayed progression to AIDS in either homozygote or heterozygote individuals.

Coreceptor-targeted anti-HIV therapy

- CXCR4 inhibitors:
  - The bicyclam AMD3100 is a well-known small molecule CXCR4 antagonist that strongly and specifically inhibits X4 HIV-1 infection. Tetrahydroquinoline-based AMD070 is a potent and specific X4 HIV-1 inhibitor with high oral bioavailability;
  - T22, an 18-mer anti-X4 HIV peptide, was designed from self-defense peptides isolated from horseshoe crabs. T140, a downsized analog of T22, has stronger anti-HIV activity than T22;
  - KRH-1636, which strongly and specifically inhibits X4 HIV-1 infection, is a novel small nonpeptide CXCR4 antagonist. KRH-3955, a derivative of KRH-1636, is much more potent than KRH-1636 and is orally bioavailable.
- CCR5 inhibitors:
  - Among the CCR5 inhibitors that have been developed, maraviroc is the only one that has been approved for use in treatment-experienced adult patients with R5 HIV-1 infection in combination with other antiretroviral drugs.

Resistance to coreceptor inhibitors

- Generation of HIV-1 strains resistant to coreceptor inhibitors *in vitro* seems difficult and time-consuming compared with generation of strains resistant to antiretrovirals that target viral proteins such as reverse transcriptase.
- Experiments using cells that expresses both CXCR4 and CCR5, such as human peripheral blood mononuclear cells, rarely result in the selection of drug-resistant viruses that change coreceptor usage.

Other applications of CXCR4 inhibitors

- Anticancer:
  - Many CXCR4 inhibitors have been shown to significantly reduce metastasis of various cancers such as ovarian carcinoma, melanoma, prostate cancer and breast cancer;
  - The CXCR4 inhibitors KRH-1636 and KRH-3955 are highly potent in antagonizing SDF-1α-mediated chemotaxis of cancer cells.
- Antiarthritis & allergy:
  - CXCR4 inhibitors AMD3100 and T140 are effective in mitigating rheumatoid arthritis and/or pulmonary fibrosis in animal models for these diseases.
- Hematopoietic stem cell mobilization:
  - AMD3100 (now known clinically as plerixafor) induces the rapid and reversible mobilization of hematopoietic stem cells from bone marrow into the circulating blood in synergy with granulocyte colony-stimulating factor.

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