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MINI-REVIEW

HIV-1 tropism

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Acquired immunodeficiency syndrome (AIDS) was reported in 1981 ((CDC), 1981). Human immunodeficiency virus type-1 (HIV-1), the virus causing AIDS, was isolated in 1983 (Barré-Sinoussi et al., 1983). Since AIDS patients were characterized by a profound decrease in CD4-positive T cell count (Gottlieb et al., 1981), it was a logical consequence that CD4 would be the most probable receptor for the virus. Actually, it was confirmed that CD4 is the primary receptor for HIV-1 in 1986 (Maddon et al., 1986). However, it was speculated that HIV-1 requires another factor to infect a cell, since CD4 alone could not support HIV-1 infection to nonhuman cells (Weiner et al., 1991). The co-receptor was an enigma for 10 years. A G protein-coupled 7 transmembrane protein, originally called fusin and currently known as CXCR4, was isolated as a co-receptor for HIV-1 in 1996 using an assay system in which fusion between Env-expressing and CD4-expressing cells leads to activation of a reporter gene (*E. coli* lacZ) (Feng et al., 1996). Interestingly, CXCR4 acted as a preferential co-receptor for HIV-1 adapted to T cell lines *in vitro*, in comparison to its activity with primary HIV-1 isolates.

Long before the identification of CXCR4 as a co-receptor, it had been known that primary HIV-1 isolates show preferential growth in either CD4-positive T cell lines (T cell-line tropic or T-tropic strains) or monocyte-derived macrophages (macrophage tropic or M-tropic strains) while they replicate well in peripheral blood mononuclear cells (PBMC) (Asjö et al., 1986; Collman et al., 1989). It was speculated that T cell lines and monocytes/macrophages carry distinct co-receptors which discriminate for T-tropic and M-tropic strains (Schuitemaker et al., 1993). β-chemokines such as RANTES, MIP-1α and MIP-1β produced by CD8-positive T cells were proven to have HIV-1-suppressive activity (Cocchi et al., 1995). RANTES, MIP-1α and MIP-1β bind toCCR5, a member of CC-chemokine receptor family, as a common receptor. CCR5 has the characteristic structure of G protein-coupled 7 transmembrane receptors. The discoveries of β-chemokines as HIV-1-suppressive agents and a molecular mimicry to CXCR4 led to a molecular identification of CCR5 as a co-receptor for M-tropic strains (Deng et al., 1996). SDF-1 was found to be a ligand of CXCR4 (Oberlin et al., 1996). A new classification for HIV-1 was proposed: R5 viruses for the isolates that use CCR5 but not CXCR4, X4 viruses for those that use CXCR4 but not CCR5 as a co-receptor (Berger et al., 1998). There are isolates that can use both CCR5 and CXCR4 and are called dualtropic.

The envelope glycoprotein of HIV-1 is synthesized as a precursor form, gp160. Trimeric gp160 molecules are transported to the Golgi apparatus, where they are cleaved by a cellular protease generating mature envelope glycoproteins: gp120, the exterior glycoprotein and gp41, the transmembrane glycoprotein (Wyatt and Sodroski, 1998). The viral determinant of the co-receptor usage had been mapped to the third variable (V3) domain of the envelope glycoprotein gp120 (O'Brien et al., 1990; Hwang et al., 1991; Shioda et al., 1991). V3 amino acids in X4 strains have a significantly higher positive charge than R5 isolates (Fouchier et al., 1992). The presence of a positively charged amino acid at 11th or 25th positions of the V3 loop is a predictive marker for the X4 phenotype (Resch et al., 2001). The high-affinity co-receptor binding site is hidden from the surface of gp120 and sequestered from the host immune surveillance (Wyatt and Sodroski, 1998). Binding to CD4 molecules induces large conformational changes in gp120 (Wu et al., 1996). The V3 loop after CD4 binding has three structural regions: a conserved base, which forms an integral portion of the gp120 core; a flexible stem, which extends away from the core; a β-hairpin tip (Huang et al., 2005). The tropism determining positions 11 and 25 are within the variable stem. This stem-β-hairpin tip structure may allow V3 to

function as a molecular hook for the co-receptor. Further conformational change in gp120 induced after co-receptor binding triggers the fusion process between viral envelope and the plasma membrane of the host cell.

It has been known that the co-receptor usage is highly related to the clinical course of HIV-1 infection. During the early stage of the infection, R5 viruses predominate. Data from cross-sectional studies show that 70%–80% of patients with early-stage disease harbor only R5 virus (Japour et al., 1995). During the natural course of the infection, viral variants that use CXCR4 appear in roughly 50% of the patients (de Roda Husman et al., 1997). It is still an enigma why R5 viruses have an advantage to replicate in the early phase of the infection. Appearance of X4 viruses is correlated often with the rapid clinical progression (Tersmette et al., 1989), which is relevant to the differential expression of CCR5 and CXCR4 according to the T cell development (Bleul et al., 1997).

Small molecular inhibitors for CCR5 and CXCR4 are available (Kuritzkes, 2009). CCR5 antagonists have been named with a suffix, "viral receptor occupancy (-viroc)." CCR5 inhibitor, maraviroc, has been approved for clinical use and vicriviroc is on the clinical trial in the resource rich countries. AMD3100 and related compounds AMD11070 have been developed as CXCR4-specific inhibitors; however, none of them have been approved for clinical use. Determinations of the co-receptor usage of patients' viruses are a prerequisite to choose maraviroc for treatment.

There are several ways to determine the HIV-1 co-receptor usage: one is the phenotype-based method and other is genotype-based (Table 1) (Koot et al., 1992; Trouplin et al., 2001; Braun and Wiesmann, 2007; Van Baelen et al., 2007). Before the co-receptors were molecularly cloned, a method to classify HIV-1 isolates according to the ability to induce syncytium formation in MT-2 cell line had been published (Koot et al., 1992). MT-2 cells express CXCR4 but not CCR5. Therefore, MT-2 assay was actually a phenotypic assay for the co-receptor usage. Virtually speaking, T-tropic viruses were syncytium-inducing (SI) and M-tropic viruses were non-syncytium inducing (NSI) viruses. More sophisticated phenotypic assays are available using reporter cell lines expressing CD4 and a co-receptor, either CCR5 or CXCR4, on the cell surface. They have been used to characterize HIV-1 present in the patients' plasma. Trofile™ (Monogram Biosciences, South San Francisco, California, USA) is one of them. HIV-1 gp160 gene in a patient's plasma is amplified, cloned into an expression vector and co-transfected with an HIV-1 genomic vector carrying a reporter luciferase gene, to generate pseudotype virions. HIV-1 tropism is judged according to the luciferase activity in the reporter cells after infection (Whitcomb et al., 2007).

Since the Trofile assay has been a single globally approved phenotypic assay for the clinical use of maraviroc, the effort to speculate the co-receptor usage from sequencing data has been continued. For example, Web PSSM (http://indra.mullins.microbiol.washington.edu/webpssm/) (Jensen et al., 2006), Geno2Pheno [coreceptor] (http://coreceptor.bioinf.mpi-inf.mpg.de/index.php) (Sing et al., 2007) and WetCat (http://genomiac2.ucsd.edu:8080/wetcat/v3.html) (Pillai et al., 2003) are algorithms to predict HIV-1 co-receptor tropism based on the V3 sequences. The V3 sequence prediction may fail to correlate with the phenotype test, since Env region other than V3 may influence the co-receptor usage (Huang et al., 2007, 2008). The sensitivity and specificity of these assays are under debate, but evidence is accumulating that the genotypic assay is useful in clinical decision-making.

HIV-1 tropism has been characterized almost exclusively on subtype B HIV-1, which is predominant in the resource rich countries. Analysis of the HIV-1 tropism in the strains predominating in Africa and Asia awaits future research. A simpler phenotypic assay system would be valuable to characterize these strains.

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Table 1 Methods currently used to determine HIV-1 coreceptor tropism

assay		Env flagment	type	detection	reference
trofile	phenotype	gp160	single cycle	Luc from the viruses	Whitcomb et al., 2007
phenoscript	phenotype	V1-V3	multiple cycle	β-Galactosiase from the cells	Trouplin et al., 2001
MT-2	phenotype	NA	multiple cycle	Syncytia formation, p24	Koot et al., 1992
tropism-testing platform	genotype and phenotype	C1-V4	multiple cycle	GFP from the viruses	Van Baelen et al., 2007
Xtrack[c]/PhenX-R	genotype and phenotype	V1-V3	multiple cycle	β-Galactosiase from the cells	Braun et al., 2007
Web PSSM	genotype	V3	NA	sequencing	Jensen et al., 2006
Geno2 Pheno [corecep-tor]	genotype	gp120	NA	sequencing	Sing et al., 2007
WetCat	genotype	V3	NA	sequencing	Pillai et al., 2003

NA, not available

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