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論文内容の要旨

The human immunodeficiency virus (HIV) is an etiological agent of acquired immunodeficiency syndrome (AIDS). The genome of HIV evolves approximately a million times faster than human genes. Because of this high mutation rate, the genomic sequences of HIVs within a single host are not homogeneous, but heterogeneous in which all members are closely related to each other. In many genes of HIV-1, preponderance of synonymous substitution over nonsynonymous substitution was observed as well as in other RNA viruses and this observation is in accordance with the neutral theory of molecular evolution. However, the third variable envelope (V3) region, one of the major epitopes of HIV-1 may be a specific site such as an antigenic site, which makes it possible to escape from the host immune system.

In this thesis, I discussed molecular evolution of HIV focusing on the following two areas;

(1) phylogenetic relationships among HIVs and related SIVs, and

(2) molecular evolution of HIV within the human body.

The origin and molecular epidemiology of human immunodeficiency virus are discussed. HIVs and SIVs (simian immunodeficiency viruses) form one group of primate lentiviruses. However, there has been a controversy over the origin of HIV and SIV. I reviewed this problem by conducting phylogenetic analyses. My phylogenetic tree clearly suggests that evolution of HIV and SIV did not follow the evolution of their host species, confirming that interspecies transmission of the virus might have occurred in the past.

Human immunodeficiency virus type 1 (HIV-1) which possibly originated in Africa is now found all over the world. Previous phylogenetic analyses showed that almost all of HIV-1 isolates from Haiti, USA and western Europe form a distinct cluster known as "subtype B." These studies suggested that one type of HIV-1 variants in Africa might have been introduced to Haiti, USA and western Europe. However, there is not much information about how HIV-1 was transmitted to India, Thailand, Romania, and Russia where the epidemiological features were reported very recently. Thus, I intended to elucidate more detailed routes of transmission of HIV-1 by phylogenetic analyses. For this purpose I collected all the available nucleotide sequences for part of the envelope glycoprotein of HIV-1 from the international DNA data bases, and I constructed phylogenetic trees using the amino acid sequences translated. I have found that HIV-1 isolates from Russia and Romania respectively form new clusters. This finding suggests that transmission of HIV-1 into Russia and Romania might have been independent of that into most developed countries such as USA and western Europe, and that geographical closeness is not necessarily correlated with similarity among the virus strains. The phylogenetic tree also supports the view that HIV-1 might have been introduced to India and Thailand directly from Africa. My phylogenetic analysis suggested that divergence of HIV-1 from Africa to India and Thailand might have started at the almost same time.

I conducted molecular evolutionary analyses of evolution of the epitope region of HIV within a single host. The third variable envelope region (V3) of HIV is known as one of the major epitopes and a determinant of the viral phenotypes of HIV. It has been suggested that positive selection may be operating on the V3 region. To elucidate the evolutionary mechanism of HIV, it is important to understand population dynamics of HIV variants within a human body. In order to understand population dynamics of HIV of the V3 region host, I analyzed the nucleotide sequence data for the V3 region of HIV clones sampled periodically from each of six patients studied. I constructed phylogenetic trees for viral variants within the human body. My phylogenetic analysis predicted particular variants that might survive AZT treatment.

I traced the change of genetic variation of the V3 region within a human body. My results also showed that genetic variations of HIV within a human body do not increase monotonically, supporting Nowak et al. 's prediction that there is a threshold in degrees of genetic diversity when the human immune system is activated to affect the dynamics of the viral population.

Moreover, I estimated the rate of nucleotide substitution using the nucleotide sequence data of HIV variants periodically sampled. Then I found that there was a certain time period after infection when the rate of nonsynonymous substitution was significantly higher than that of synonymous substitution within a human body. However, I also noticed that for other periods, the rate of synonymous substitution was higher than that of nonsynonymous substitution. Thus, the type of selection and its intensity may not be constant all the time in viral evolution within the human body.

I analyzed the patterns of amino acid substitutions at each amino acid site for the V3 region. As a result, I found that amino acid substitutions occurred predominantly at five specific amino acid sites where the substitution is known to be responsible for production of antigenic variation and determination of viral phenotypes. These findings indicate a strong possibility that positive selection is taking place in the V3 region of HIV during a particular time period after infection.

My analyses showed that highly variable amino acid sites are responsible not only for antigenicity but also for cell tropism. This indicates that positive selection may also operate on mutations that alter viral cell tropism and replication rates. Many of the amino acid substitutions occurring in the V3 region might enable the virus to escape from recognition by the host immune system or cause a successful increase in population frequencies by changing viral phenotypes. In particular, during the periods of time when nonsynonymous substitutions predominate, HIV may change its antigenicity or phenotype associated with virulence.

論文の審査結果の要旨

山口由美さんは、エイズウイルスであるHIV(human immunodeficiency virus)の分子進化学的研究を行った。HIV の塩基置換速度は、宿主のゲノムより１００万倍程度高く、一人の感染者の体内に存在するクローン間においても多くの変異が存在することが知られ、経時的に採られたクローンの塩基配列データが解析可能であった。山口さんは、HIVの起源と伝播過程、HIVの宿主の体内での進化、という２つの視点で研究を行った。

HIV-1の世界各地への伝播過程を明らかにする目的で、世界各地から単離されたHIVの塩基配列を国際DNAデータベースから集め、近隣結合法で分子系統樹を作成している。その結果、ロシアとルーマニアから単離されたHIV-1株は、西ヨーロッパからの単離株によりも、アフリカ大陸で単離された株に近いことを見出し、伝播過程に関する新知見を得た。

HIVの外被糖蛋白質の高変異領域であるV3領域は、主要な抗原決定基であり、V3領域のアミノ酸配列の変化が、抗体との結合に影響し、細胞親和性をも変化させる。山口さんは、この領域の宿主内進化を解明する目的で、宿主の体内においてV3領域の異変が経時的に調べられている６人の患者の塩基配列の分子系統樹を作成した。６人の患者とも、はじめは体内でほぼ均一であったV3領域の塩基配列が、時間が経過するにつれて塩基置換を蓄積して遺伝的に区別できる系統に分かれる過程を明らかとした。一例の患者では、他の患者と同様に、時間と共に塩基配列の変異が増大する傾向にあったが、AZT投与直後に採られたウイルス株は互いに非常に近縁であり、塩基配列の多様性の減少を見出している。AZTの影響を受けて、特定の変異体のみが生き延びた可能性を指摘している。

複数の患者において、非同義置換速度が同義置換速度を大きく上回る時期の存在したことを見出し、V3領域が宿主の免疫系の影響を受けつつ、適応的に進化する時期が存在した可能性を指摘している。また、V3領域のアミノ酸座位ごとに患者の体内で生じた置換数を推定して、抗体による認識部位に、アミノ酸置換が集中することをも明らかにした。これらの解析結果を基礎に、V3領域が宿主の免疫系などの影響を受けつつ、ウイルスの増殖に有利な変異を蓄積して、適応的に進化している分子進化過程を提唱している。この研究は、HIVウイルスの進化に関する新知見をもたらすものであり、エイズの治療法の開発にも役立つ可能性が考えられ、学位論文としての条件を充分に満たすと判断した。