Mapping the domains of CD134 as a functional receptor for feline immunodeficiency virus (FIV)

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ABSTRACT

The feline homologue of CD134 (fCD134) is the primary binding receptor for feline immunodeficiency virus (FIV), targeting the virus preferentially to activated CD4+ helper T cells. However, strains of FIV differ in their utilisation of CD134; the prototypic strain PPR, requires a minimal determinant in CRD1 of fCD134 to confer near optimal receptor function while strains such as GL8 require additional determinants in the CD134 CRD2. We map this determinant to a loop in CRD2 governing the interaction between the receptor and its ligand; substitution of amino acids S78N,S79Y,K80E restored full viral receptor activity to the CDR2 of human CD134 in the context of feline CD134 with tyrosine-79 appearing to be the critical residue for restoration of receptor function.

The initial event in the process of viral entry into a target cell is the interaction between the virus and its cellular receptor and the specificity of this interaction determines both the cell tropism and the pathogenicity of the virus. The primary receptor for FIV is CD134 (OX40)[13], a member of the tumour necrosis factor receptor/nerve growth factor receptor (TNFR/NGFR) family of molecules. CD134 expression is greatest on activated CD4+ T-helper cells[7] thus infection results in a progressive depletion of T-helper cells an acquired immune deficiency syndrome (AIDS)-like illness. Primary isolates of FIV use CD134 as the binding receptor in conjunction with the chemokine receptor CXCR4 as a co-factor for infection[11,13,15]. FIV binds specifically to CD134-expressing cells[13], the FIV Env interacts directly with CD134[4] and pre-treatment of virus with sCD134 facilitates infection of CD134-ve / CXCR4+ve cells[3].

The binding site for the PPR strain of FIV has been mapped to the first cysteine-rich domain (CRD1)[3]; human CD134 is not a functional receptor for FIV[13] and substitution of CRD1 of human CD134 with that of feline CD134 renders the molecule functional as a receptor for the PPR strain of FIV. However expression of feline CD134 CRD1 in the context of human CD134 is insufficient to confer receptor function on many primary strains of FIV, additional determinants in CRD2 of feline CD134 being required to restore function[16], indicating differential utilization of CD134 by diverse strains of FIV[16]. In order to map the determinants in CDR2, we compared the amino acid sequences of the CRD2s of feline and human CD134 (previously we identified the fragment spanning amino acids 65 to 82 as containing the critical determinant(s)[16]) and identified amino acid sequence differences (Fig. 1b). Next, using the human CRD2 containing chimaera FFHH as our template, we proceeded to mutate the remaining amino acids in CRD2 from the human sequence to

the feline. The following mutations were introduced: R66L, P67Q (RP>LQ); G69A, P70S (GP>AS); D75E,V76A (DV>EA), S78N, K80E (SK>NE), and S78N, S79Y, K80E (SSK>NYE). The nucleic acid sequence of each mutant was confirmed; the constructs were then cloned into the retroviral pDONAI and packaged into MLV pseudotypes as previous[16] and then used to transduce HeLa cells. Stable transductants were selected in G418 and surface expression of the chimaeric receptors confirmed by flow cytometry using the BerACT35 monoclonal antibody. Flow cytometric analysis confirmed that following G418 selection, the constructs were expressed at comparable levels at the cell surface (not shown).

The stable cell lines were then seeded into 96-well plates and infected with HIV(FIV) pseudotypes bearing FIV Envs identified previously as displaying a marked preference for chimaera FHFH (amino acids 65 to 82 feline in origin) over chimaera FFHH (amino acids 65 to 82 human in origin); Envs GL8, CPG41 and 0827, or displaying no or little preference between FHFH and FFHH; Envs 1419, B2542 and PPR[16]. Each of the viral pseudotypes infected parent feline CD134 (FFF)-expressing cells and the FHFH chimaera-expressing cells with similar efficiency while human CD134 (HHH)-expressing cells did not differ significantly from control HeLa (CON) transduced with vector only. Introduction of the R66L, P67Q, G69A, P70S and D75E, V76A substitutions into the FFHH chimaera did not render the molecule a functional receptor for GL8, CPG41 and 0827 Env-bearing pseudotypes. S78N, K80E restored partial function for CPG41 and 0827 Envs while S78N, S79Y, K80E restored full viral entry receptor function to FFHH for infection with GL8, CPG41 and 0827-Env bearing pseudotypes. The data suggest that the NYE (Asn78-Tyr79-Glu80) motif in feline CD134 CRD2 is a critical determinant of receptor function for viruses such as GL8, CPG41 and 0827 and of these residues, Y79 would appear to be the most

important. Although the 1419 and B2542 strains showed only weak selectivity for usage of FHFH over FFHH (<10-fold), the S78N, S79Y, K80E mutation restored full functionality. In contrast, the PPR strain was at the other extreme from the GL8, CPG41 and 0827 strains, utilising all chimaeras with similar efficiency.

These findings may indicate that the PPR/CD134 interaction is more relaxed than the interactions with the GL8, CPG41 and 0827 strains. This may be the result of either a higher tolerance of the PPR Env for amino acid changes within CRD2 or that in contrast to GL8, CPG41 and 0827, PPR Env binding to CD134 does not involve CRD2. Consistent with this hypothesis, the binding site for FIV PPR Env has been mapped to CRD1 of feline CD134 in the region encompassing residues D60 and D62[3]. If the structural predictions for feline CD134[3,16] prove to be accurate, then the determinant identified in this study lies in CRD2 on the opposite face of the molecule from the Env binding site (Figure 2a). Tyr79 sits at the crown of a loop defined previously as the "A1 module" of an A1B2 cysteine-rich domain[2] (CRD2). The crystal structures of the TNF/TNFR[1] and Apo2L/DR5[6] complexes place this loop at the ligand-receptor interface (Fig. 2b). Comparison of the structural predictions for feline and human CD134 illustrate that feline CD134 would present a bulky tyrosine residue (Tyr79) at the ligand binding face of the receptor and that this would be flanked by a negatively-charged glutamate residue (E80). In contrast, human CD134 would have a smaller serine residue (Ser79) flanked by a positively-charged lysine (Lys80). We have constructed the following models to account for the effect that substitution of residues 78NYE80 in feline CD134 with 78SSK80 has on the function of feline CD134 as a viral receptor:

i. Allosteric effect. An allosteric effect on the binding domain in CRD1, disrupting recognition of the domain by the GL8, CPG41 and 0827 Envs. Previous studies on

the interaction between herpes simplex virus-1 (HSV-1) gD and its receptor HveA (herpes simplex virus entry mediator A) revealed that binding of the HveA ligands LT-α (lymphotoxin-A) or LIGHT to HveA perturbs its function as a receptor for HSV-1. Conversely, binding of HSV-1 gD to HveA inhibits the interaction between HveA and LIGHT[9]. As HSV-1 gD and LT-α/LIGHT bind to distinct sites on HveA[12], the data indicated that ligand binding to HveA altered the conformation of the receptor, perturbing its function. By analogy with HveA, the 78NYE80 region in feline CD134 may be required for maintenance of the Env-binding domain (in CRD1) and the NYE>SSK substitution alters the conformation of the virus binding domain sufficiently to ablate recognition by the GL8, CPG41 and 0827 Envs but not PPR which in turn must recognise a minimal (non-conformation-dependent) determinant. Accordingly, soluble CRD1 of CD134 does not activate FIV Env for a subsequent interaction with CXCR4 while a soluble form of the entire extracellular domain of CD134 facilitates the interaction efficiently[5].

ii. Perturbation of the CD134/CD134L interaction. Residues ^{78}NYE^{80} in feline CD134 may be critical for the interaction between CD134 and CD134L, the A1 module of CRD2[2] having been shown previously to lie at the receptor-ligand interface for TNF/TNFR[1] and Apo2L/DR5[6]. Alignment of the amino acid sequences of feline and human CD134 with TNFR1 and DR5 (Fig. 2c) using the Clustal algorithm places 78NYE80 in same location as the residues of TNFR1 and DR5 CRD2s that are buried in the receptor-ligand interface (Fig 2c.). Thus it is likely that residues 78NYE80 are involved in the CD134/CD134L interaction and the NYE>SSK substitution may disrupt this interaction. In the absence of soluble feline CD134L, we assessed the binding of soluble human CD134L (shCD134L) to MCC cells expressing FFHH and FHFH chimaeras (Fig. 2d.). FFHH bound shCD134L efficiently (63.0%

positive at 10ng/ml, 97.6% at 100ng/ml), consistent with this chimaera containing the entire CRD2 of human CD134 and CRD2 governing ligand binding. FHFH bound shCD134L less efficiently (60.1% at 100ng/ml) consistent with amino acids 65 to 82 of feline CD134 in CRD2 disrupting ligand binding. Binding of shCD134L to fCD134-expressing MCC cells did not differ significantly from binding to control MCC cells (18.6% and 15.1% respectively at 100ng/ml). Thus, mutations in the A1 loop of CRD2 disrupt binding of shCD134L to the CRD2 of hCD134. Accordingly, it is possible that the NYE>SSK substitution disrupts the receptor-ligand interaction and in doing so, prevents recognition of a multimeric form of the receptor by the GL8, CPG41 and 0827 Envs. Further dissection of the role of the receptor-ligand interaction in viral receptor function will require the cloning and expression of functional feline CD134L. However, it is notable that cell lines known to be highly susceptible to FIV express not only CXCR4 and CD134, but also CD134L (Table 1).

Given that the interaction between the GL8, CPG41 and 0827 Envs would appear to be complex and involve regions of the CRD2 of CD134 predicted to lie at the receptor-ligand interface, our studies raise the possibility that infection by such viruses may well be modulated (inhibited or enhanced) by the natural ligand for feline CD134 (CD134L or OX40 ligand).

The biological significance of differences in the affinity of virus-receptor interaction has been revealed by studies on the adaptation of HIV for growth in cell culture; primary strains displaying differential requirements for levels of CD4 and CXCR4[14]. These findings are mirrored by analyses of the molecular determinants of viral growth in microglia, where microglia-tropic strains utilise CD4 more efficiently by way of a higher affinity Env-CD4 interaction and increased exposure of the chemokine receptor binding site[8]. Further, a high affinity interaction between the

prCBL23 Env and CD4 may mediate Lv2 restriction[10]. Revealing the biological significance of the differential utilization of CD134 by FIVs may inform the design of novel strategies for vaccination and therapy and further strengthen the comparative value of FIV infection of the domestic cat as a non-primate model for HIV and AIDS.

Figure 1. (a.) Schematic representation of the FFHH and FHFH CD134 chimaeras[16] highlighting FIV PPR binding site[3], tyrosine-79 (Y79) and serine-79 (S79). (b.) Amino acid sequence comparison of the region spanning CRD1 and CRD2 of CD134. Divergent amino acids in region 65 to 85 targeted for mutagenesis are shaded. (c.) HeLa cells transduced with CD134 mutants and stably selected in G418 were infected with HIV (FIV) luciferase pseudotypes bearing GL8, CPG41, 0827, 1419, B2542 and PPR Envs. Luciferase activity (CPM/ml) was quantified 72 hrs post-infection, each bar represents mean +/- SE, n=3.

Figure 2. (a.) Predicted 3D-structures of feline and human CD134 showing the locations of the FIV-PPR SU binding site (59GDQD62[3], "BS") and the 78NYE80 and 78SSK80 regions of feline and human CD134 respectively. (b.) Location of H66, L67 and R68 (space-filling spheres) on TNFR (c.) Amino acid sequence comparison of the A1 modules of the CRD2s of feline CD134, human CD134, TNFR1, DR5 (Apo2) and Fas. 78NYE80 and 78SSK80 are boxed. Residues that bury more than 50% of their accessible surface area in the TNF/TNFR and Apo2L/DR5 receptor-ligand interface[6] are shown in bold. (d.) Flow cytometric analysis of binding of shCD134L to MCC cells expressing fCD134 or the chimaeras FFHH and FHFH. shCD134L (R&D systems) was incubated at 4^{o}C for 30 mins. before detection with 0.1μg goat anti-human CD134L (R&D systems) followed by FITC-conjugated rabbit anti-goat IgG (Sigma). 10,000 events were collected per sample.

Figure 1.

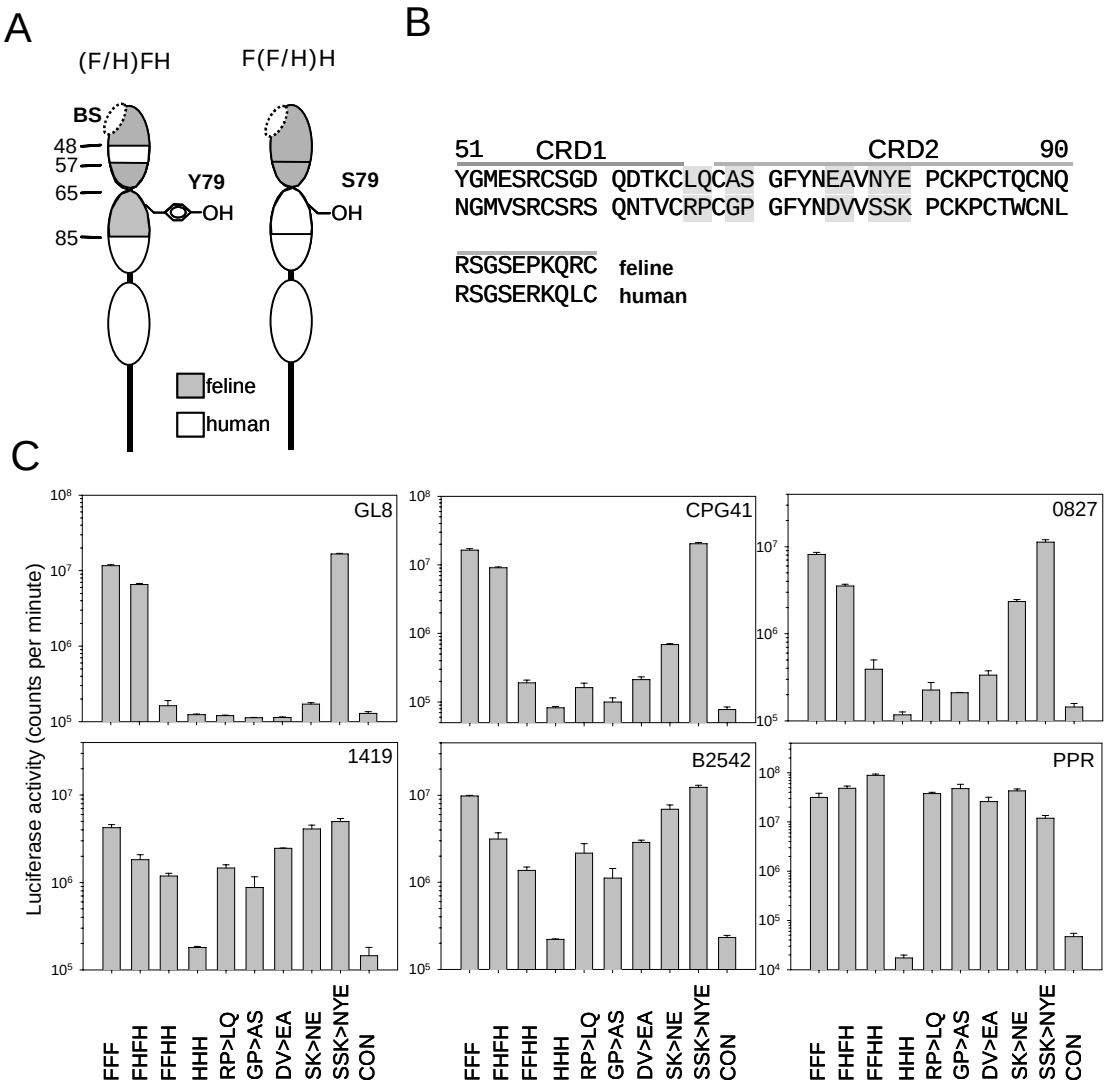
A
(F/H)FH
F(F/H)H
BS
48
57
65
85
Y79
OH
S79
OH
feline
human
B
51 CRD1 CRD2 90
YGMESRCSGD QDTKCLQCAS GFYNEAVNYE PCKPCTQCNQ
NGMVSRCSRS QNTVCRPCGP GFYNDVVSSK PCKPCTWCNL
RSGSEPKQRC feline
RSGSERKQLC human
C
Luciferase activity (counts per minute)
GL8
CPG41
0827
1419
B2542
PPR
FFF
FHFH
FFHH
HHH
RP>LQ
GP>AS
DV>EA
SK>NE
SSK>NYE
CON

Figure 2.

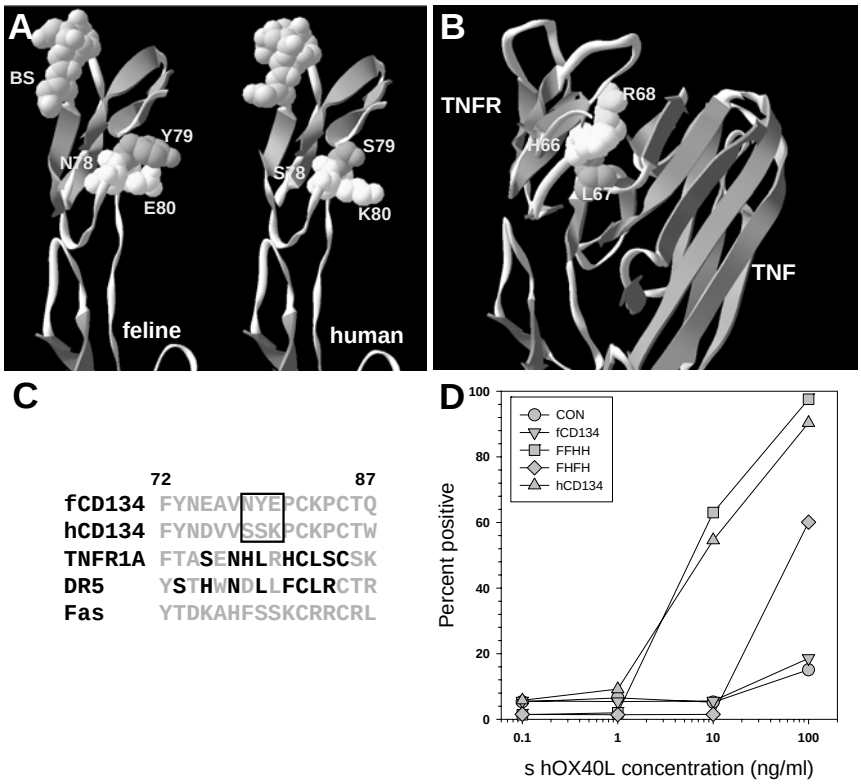
A
BS
Y79
N78
E80
S79
S78
K80
feline
human
B
TNFR
R68
H66
L67
TNF
C
72
87
fCD134 FYNEAVNYEPCKPCTQ
hCD134 FYNDVVSSKPCKPCTW
TNFR1A FTASENHLRHCLSCSK
DR5 YSTHWNDLLFCLRCTR
Fas YTDKAHFSSKCRRCRL
D
CON
fCD134
FFHH
FHFH
hCD134
Percent positive
100
80
60
40
20
0
0.1
1
10
100
s hOX40L concentration (ng/ml)

Table 1. Real time PCR measurement of CXCR4, CD134 and CD134L. Measurement of CD134, CD134L and CXCR4 gene expression by real time reverse transcription (RT) PCR. Total RNA was extracted from MBM and MYA T-lymphoid cell lines, reverse transcribed into cDNA using random hexamers, and amplified for feline CD134, CD134L and CXCR4 and housekeeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as internal control (Pistello et al., submitted). Primers and probes are available by e-mail on request. Relative gene expression is given as the difference between the threshold cycle (C_T) of the target and the C_T of internal control (ΔC_T).

Sample description	GAPDH	CD134		CD134L		CXCR4	
	Ct	Ct	ΔCt	Ct	ΔCt	Ct	ΔCt
MBM	20.14	20.46	-0.32	28.52	-8.38	23.66	-3.52
MYA-1	18.44	18.27	0.17	23.50	-5.06	22.43	-3.99

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R**EFERENCES**

1. **Banner, D. W., A. D'Arcy, W. Janes, R. Gentz, H. J. Schoenfeld, C. Broger, H. Loetscher, and W. Lesslauer**. 1993. Crystal structure of the soluble human 55 kd TNF receptor-human TNF beta complex: implications for TNF receptor activation. Cell **73**:431-445.

2. **Bodmer, J. L., P. Schneider, and J. Tschopp**. 2002. The molecular architecture of the TNF superfamily. Trends Biochem. Sci. **27**:19-26.

3. **de Parseval, A., U. Chatterji, G. Morris, P. Sun, A. J. Olson, and J. H. Elder**. 2004. Structural mapping of CD134 residues critical for interaction with feline immunodeficiency virus. Nat. Struct. Mol. Biol.

4. **de Parseval, A., U. Chatterji, P. Sun, and J. H. Elder**. 2004. Feline immunodeficiency virus targets activated CD4+ T cells by using CD134 as a binding receptor. Proc. Natl. Acad. Sci. U. S. A **101**:13044-13049.

5. **de Parseval, A., C. K. Grant, K. J. Sastry, and J. H. Elder**. 2006. Sequential CD134-CXCR4 interactions in feline immunodeficiency virus (FIV): soluble CD134 activates FIV Env for CXCR4-dependent entry and reveals a cryptic neutralization epitope. J. Virol. **80**:3088-3091.

6. **Hymowitz, S. G., H. W. Christinger, G. Fuh, M. Ultsch, M. O'Connell, R. F. Kelley, A. Ashkenazi, and A. M. de Vos**. 1999. Triggering cell death: the crystal structure of Apo2L/TRAIL in a complex with death receptor 5. Mol. Cell **4**:563-571.

7. **Mallett, S., S. Fossum, and A. N. Barclay**. 1990. Characterization of the MRC OX40 antigen of activated CD4 positive T lymphocytes--a molecule related to nerve growth factor receptor. EMBO J. **9**:1063-1068.

8. **Martin-Garcia, J., W. Cao, A. Varela-Rohena, M. L. Plassmeyer, and F. Gonzalez-Scarano**. 2006. HIV-1 tropism for the central nervous system: Brain-derived envelope glycoproteins with lower CD4 dependence and reduced sensitivity to a fusion inhibitor. Virology **346**:169-179.

9. **Mauri, D. N., R. Ebner, R. I. Montgomery, K. D. Kochel, T. C. Cheung, G. L. Yu, S. Ruben, M. Murphy, R. J. Eisenberg, G. H. Cohen, P. G. Spear, and C. F. Ware**. 1998. LIGHT, a new member of the TNF superfamily, and lymphotoxin alpha are ligands for herpesvirus entry mediator. Immunity. **8**:21-30.

10. **Reuter, S., P. Kaumanns, S. B. Buschhorn, and M. T. Dittmar**. 2005. Role of HIV-2 envelope in Lv2-mediated restriction. Virology **332**:347-358.

11. **Richardson, J., G. Pancino, T. Leste-Lasserre, J. Schneider-Mergener, M. Alizon, P. Sonigo, and N. Heveker**. 1999. Shared usage of the chemokine receptor CXCR4 by primary and laboratory-adapted strains of feline immunodeficiency virus. J. Virol. **73**:3661-3671.

12. **Sarrias, M. R., J. C. Whitbeck, I. Rooney, C. F. Ware, R. J. Eisenberg, G. H. Cohen, and J. D. Lambris**. 2000. The three HveA receptor ligands, gD, LT-alpha and LIGHT bind to distinct sites on HveA. Mol. Immunol. **37**:665-673.

13. **Shimojima, M., T. Miyazawa, Y. Ikeda, E. L. McMonagle, H. Haining, H. Akashi, Y. Takeuchi, M. J. Hosie, and B. J. Willett**. 2004. Use of CD134 as a primary receptor by the feline immunodeficiency virus. Science **303**:1192-1195.

14. **Tokunaga, K., M. L. Greenberg, M. A. Morse, R. I. Cumming, H. K. Lyerly, and B. R. Cullen**. 2001. Molecular basis for cell tropism of CXCR4-dependent human immunodeficiency virus type 1 isolates. J. Virol. **75**:6776-6785.

15. **Willett, B. J., M. J. Hosie, J. C. Neil, J. D. Turner, and J. A. Hoxie**. 1997. Common mechanism of infection by lentiviruses. Nature **385**:587.

16. **Willett, B. J., E. L. McMonagle, S. Ridha, and M. J. Hosie**. 2006. Differential utilization of CD134 as a functional receptor by diverse strains of feline immunodeficiency virus (FIV). J. Virol. **80**:3386-3394.