Regulation of Human Gene of Influenza A Virus-Associated NS1-Binding Protein by 2,3,7,8-TCDD: Mechanistic Data and Epidemiological Findings

Jerry L. Wu[1], Andre G. Pokrovsky[2], Vladimir S. Rumak[3], Ilya B. Tsyrlov[1]

[1]XENOTOX Inc., Scarsdale, U.S.A. (e-mail: xenotoxit@optonline.net). [2]Novosibirsk State University, Russia.

[3]Research Tropical Center, Hanoi, Vietnam

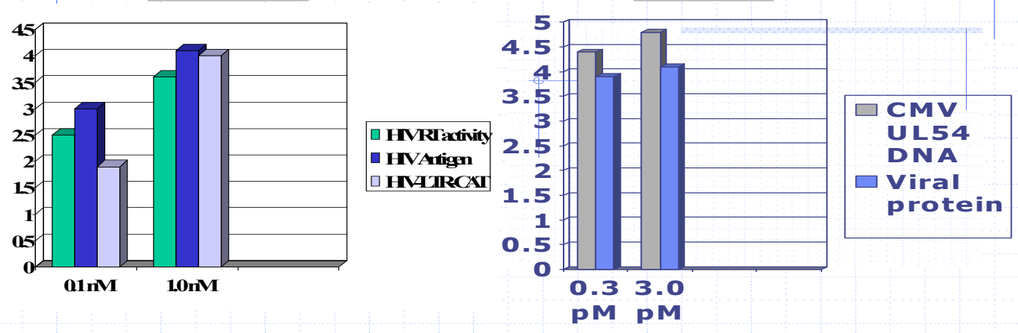
Earlier we postulated that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) might transactivate HIV-1 gene expression in HIV-infected human cells. The results have been confirmed in cell models with HIV-1 and CMV, and a clinical study on HIV-AIDS patients.

Ah receptor (AhR)-mediated transcriptional response network was shown in HIV-1 and CMV activation by TCDD, however, mechanistic concept was formulated after identifying "dioxin response elements" (DRE), core nucleotide sequence 3' A-CGCAC 5', in promoter distal region of HIV-1, CMV and several cancer-associated common human viruses.

Here, the above mechanistic concept is applied to genetically similar genes encoding human and chicken influenza virus NS1 binding protein (NS1BP), as two DREs were revealed in 5'-flanking region (at positions - 7942 and - 687).

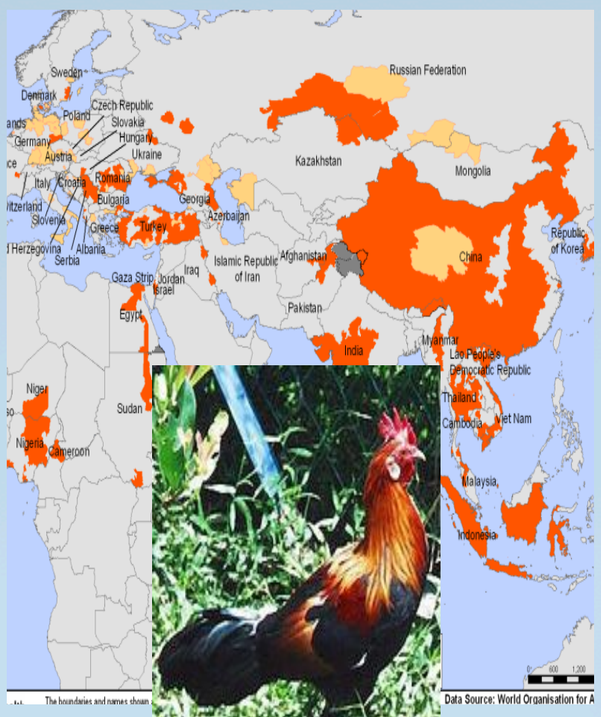
PREVIOUS DATA ON HUMAN VIRUSES

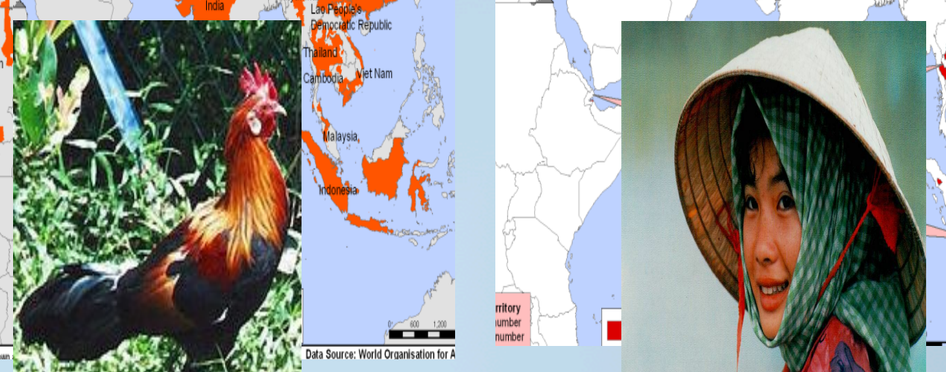
TCDD trans-activates replication of human viruses



From: Pokrovsky, Tsyrlov et al., *BBRC* 1991; Tsyrlov & Pokrovsky, *Xenobiotica* 1993; Gollapudi et al., *BBRC* 1996; Murayama et al., *BBRC* 2002; Ohata et al., *Microbiol. Immunol.* 2003; Tsyrlov, *Organohalogen Compounds* 2006

EPIDEMIOLOGIC CONCEPT





Gallus gallus **versus** ***Homo sapiens***

Influenza Virus A NS1 BP Genomic Alignment:

DREs IN PROMOTER REGION OF VIRUSES AND NS1BP

Organism summary of the dioxin response element (DRE) core sequence (5'-GCGTG-3') found in viral promoters in the Eukaryotic Promoter Database [from T. Zacharewski, 2002]

Species	# DREs Located	# Promoters Represented
Human adenovirus type 12	10	4
Human adenovirus type 2	36	9
Human adenovirus type 5	19	5
Human adenovirus type 7	12	5
Simian adenovirus (7P)	3	1
Human herpesvirus 4	154	22
Hepadnavirus		
Duck hepatitis B virus	6	2
Human hepatitis B virus	4	4
Human cytomegalovirus	102	10
Human herpes simplex virus type 1	345	30
Human herpes simplex virus type 2	38	8
Murine cytomegalovirus	1	1
Papillomavirus		
Bovine papillomavirus type 1	15	6
Human Papillomavirus type 16	3	1
Human Papillomavirus type 18	9	2
Polyomavirus		
Mouse polyoma virus	1	1
Simian virus 40	5	3
Parvovirus		
(Murine) parvovirus H1	4	2
Adeno-associated virus 2	9	3
Retrovirus		
Human immunodeficiency virus type 1	1	1
Human immunodeficiency virus type 2	2	1
Simian AIDS retrovirus SRV-1	3	1
Sarcoma virus		
(Avian) Rous sarcoma virus	7	1
Bovine leukemia virus	1	1
Gibbon ape leukemia virus	1	1
Human T-cell leukemia virus type I	4	1

Positions of Dioxin Response Elements (DRE) in IVNS1ABP Gene

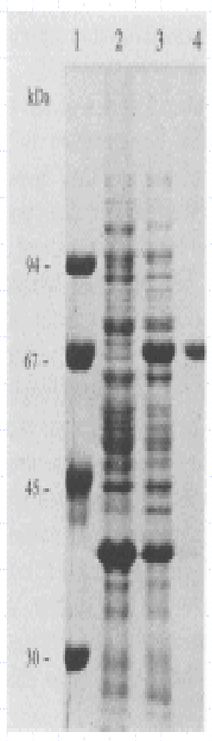
Genomic Information:
Gene title: influenza virus NS1A binding protein
Genomic full length in this probe set: 19,702 bp
Exon number: 15

Type	Length [bp]	DRE number	DRE Position
5'-Flanking	10,000	2	-7942, -687
Exon 1	203	0	
Intron 1	4,619	2	410, 1445
Exon 2	229	0	
Intron 2	1,376	1	969
Exon 3	130	0	
Intron 3	443	0	
Exon 4	173	0	
Intron 4	105	0	
Exon 5	78	0	
Intron 5	2,013	0	
Exon 6	174	0	
Intron 6	539	1	376
Exon 7	129	0	
Intron 7	1,368	0	
Exon 8	129	0	
Intron 8	3,363	4	304, 1237,1353,2211
Exon 9	131	0	
Intron 9	200	0	
Exon 10	225	0	
Intron 10	539	0	
Exon 11	122	0	
Intron 11	91	0	
Exon 12	127	0	
Intron 12	83	0	
Exon 13	133	0	
Intron 13	186	0	
Exon 14	174	0	
Intron 14	1,476	0	
Exon 15	1,154	1	
3'-Flanking	10,000	2	351, 2041

OVERLAPPING SPOTS OF H5N1 OUTBREAKS AND DIOXIN CONTAMINATION IN CHINA AND VIETNAM

NEW FINDINGS ON TCDD-NS1BP

Immunoblotting of TCDD-Induced human NS1BP



Confluent monolayers of HeLa cells, pretreated with 0.1 nM TCDD for 36 h (column 3), were lysed in RIA-precipitation assay buffer. Soluble proteins from equivalent volumes of extract corresponding to 10^4 cells were separated by SDS gel electrophoresis, transferred to a nitrocellulose membrane, and probed with affinity-purified NS1BP-specific antibodies. The marker proteins - column 1; non-treated HeLa cells – column 2; the recombinant NS1BP – column 4.

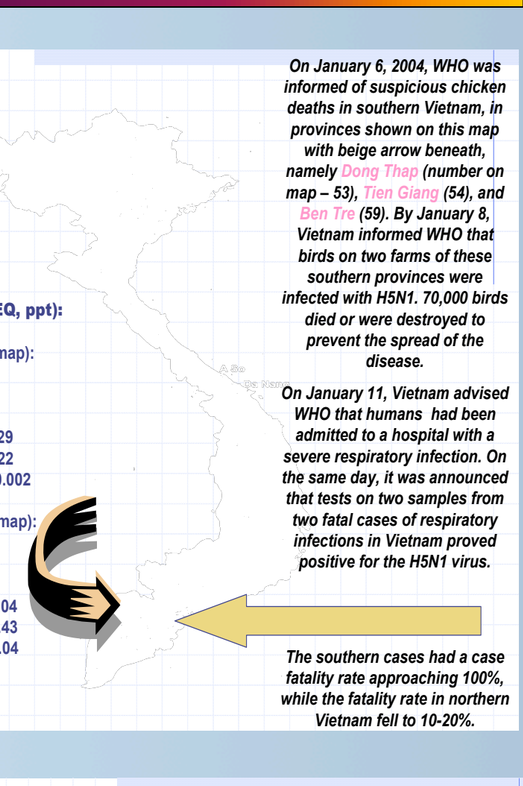
Dioxin Exposure Level in Guangdong Province, China's First H5N1 Outbreak

"The Asian H5N1 virus was first detected in Guangdong Province, China, in 1996, but it received little attention until it spread through live-poultry markets in Hong Kong to humans in May 1997, killing 6 of 18 infected persons. From 1997 to May 2005, H5N1 viruses were largely confined to Southeast Asia, but after they had infected wild birds in Qinghai Lake, China, they rapidly spread westward".
(From: Regional WHO director Dr. S. Omi & Dr. R. Webster)

"According to the Chinese Ministry of Health, human bird flu infection confirmed in Guangdong Province. A 32-year-old man died of bird flu infection, H5N1. The man was from the province of Guangdong".
(From C. Nordqvist, Med New Today, March 6, 2006)

"Mothers born in Chinese Guangdong province, have had a significantly higher CALUX-TEQ (a luciferase expression bioassay for maternal exposure to dioxin and dioxin body load). Higher seafood consumption was associated with a higher maternal CALUX-TEQ level".
(From: E. Nelson et al., Environ Sci Technol 40:1432-38, 2006)

Content of Dioxins in Humans and Chicken from Non-Treated Northern (■) and Agent Orange-Treated Southern (■) Regions of Vietnam (2005)



Dioxin Level (TCDD-based TEQ, ppt):

Northern Provinces (number on map):
- Ha Noi (17)
- Thang Hoa (27)
- Nghe An (28)

Human blood (n = 168): 1.20 ± 0.29
Chicken fat tissues: 2.02 ± 0.22
Chicken eggs: 0.015 ± 0.002

Southern Provinces (number on map):
- Tay Ninh (48)
- Binh Duong (49)
- Can Tho (60)

Human blood (n = 1210): 3.53 ± 1.04
Chicken fat tissues: 3.13 ± 0.43
Chicken eggs: 0.35 ± 0.04

On January 6, 2004, WHO was informed of suspicious chicken deaths in southern Vietnam, in provinces shown on this map with beige arrow beneath, namely Dong Thap (number on map – 53), Tien Giang (54), and Ben Tre (59). By January 8, Vietnam informed WHO that birds on two farms of these southern provinces were infected with H5N1. 70,000 birds died or were destroyed to prevent the spread of the disease.

On January 11, Vietnam advised WHO that humans had been admitted to a hospital with a severe respiratory infection. On the same day, it was announced that tests on two samples from two fatal cases of respiratory infections in Vietnam proved positive for the H5N1 virus.

The southern cases had a case fatality rate approaching 100%, while the fatality rate in northern Vietnam fell to 10-20%.

MECHANISTIC CONCEPT

Three steps body burden TCDD might facilitate flu viremia:

Transactivation of NS1BP gene thru promoter DRE (1); Physical association of NS1BP (ARA3) with AhR (2); antiviral defense-dismantling interaction of induced NS1BP with viral NS1 (3)

(1) Current data on NS1 gene promoter DRE, and immunoblotting of TCDD-induced human NS1BP

(2) "The aryl hydrocarbon receptor signaling pathway is modified through interactions with a Kelch protein", by Dunham et al., *Mol Pharmacol* 70:8-15, 2006

(3) "NS1-binding protein (NS1-BP): a novel human protein that interacts with influenza A virus nonstructural NS1 protein is relocated in the nuclei of infected cells", by Wolff et al. *J Virol* 72:7170-80, 1998

ESTIMATED RELATIVE SUSCEPTIBILITY to TCDD of VIRAL (*HIV-1* and *CMV*) and HUMAN (*NS1BP and CYP1A1*) GENES

Name (symbol) of the Gene	*HIV-1*	*CMV*	*NS1BP*	*CYP1A1*
Quantity of DRE(s) in gene enhancer	1	10	2	2
Minimal gene - activating concentration of TCDD	0.1 nM or 32 ng/kg	0.3 pM or 0.1 ng/kg	33 pM or 10 ng/kg	33 pM or 10 ng/kg

CONCLUDING REMARKS

The above information on domestic poultry dying from H5N1 in Guangdong province of China, and Long An, Tieng Giang and Ben Tre provinces of Vietnam might relate to the fact that water and soil in these very regions in China and Vietnam are highly contaminated with dioxin-like compounds.

As human *NS1BP* gene promoter contains two DRE, an extrapolation from the data on *HIV-1* (1 DRE) and *CMV* (10 DRE) genes suggested that TCDD level upregulating *NS1BP* gene should be moderately above current TCDD blood levels in general population. The data revealed here show that NS1BP might be induced in human HeLa cells with 0.1 nM TCDD. The same concentration of TCCD has been shown earlier to induce human microsomal cytochrome P4501A1, encoded by *CYP1A1* gene, the promoter region of which also possesses two DRE.

Thus in human subgroups slightly exposed to TCDD, its body level is a possible promotional factor for seasonal influenza virus A outbreaks, and may strongly facilitate spreading of the H5N1 if avian flu pandemic were to occur. This is because the resistance of highly virulent H5N1 to antiviral effects of IFN-ß and TNF-α is directly associated with viral NS1.

Non-structural protein 1

NS1, a dimeric protein consisting of six helical folds, is encoded by the influenza A viruses. NS1 is a RNA-binding protein required for virus replication. Its RNA-binding activities are:

NS1binds to the poly A tails of cellular pre-mRNAs. Thus it retains these pre-mRNAs within the cell's nucleus by blocking their export into the cytoplasm.

NS1 blocks pre-mRNA splicing by binding to a stem-buldge in U6 small nuclear RNA (snRNA) thus inactivating any U6-U2 or U6-U4 interactions that are required for splicing.

NS1 can bind double-stranded RNA (dsRNA) with a low sequence specificity while maintaining a high affinity which blocks the activation of PKR kinase.

Gene Symbol : IVNS1ABP (humans)

Homologs: IVNS1ABP (G. Gallus gallus), MGI:2152389 (Mice)LOC457584 (Pan troglodytes), etc.

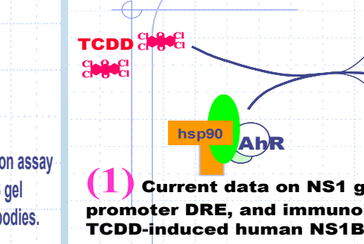
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo. etc.

The Recombinant Protein Molecular Weight (Da): 71729

Gene Map Locus: 1q25.1-q31.1

Localization: Nucleoplasm

Domains: BTB (32-129), KELTCH (369-415), KELTCH (416-463), KELTCH (464-512), KELTCH (513-559), KELTCH (650-606), KELTCH (607-641)



Expression: Brain, Heart, Kidney, Liver, Lung, Ovary, Pancreas, Muscle, Spleen, Testis, Plasma

Functions: Protein binding; Response to virus; Transcription factor complex; RNA splicing; Spliceosome complex; Transcription from Pol III promoter

printed by
MegaPrint INC.
www.postersession.com