Pathogenesis and Immuno-control of Singapore Grouper Iridovirus (SGIV)

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Groupers are major high-value species being cultured in China and Southeast Asia countries. They are particular popular for the live fish market mainly in Hong Kong, Taiwan, main land of China and SE countries

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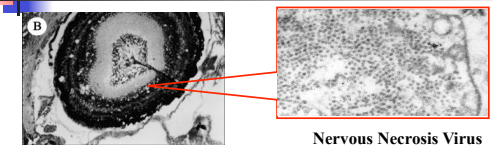
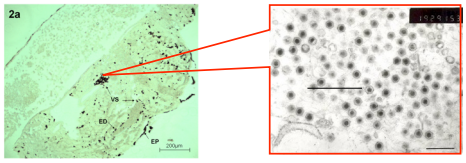
The cultured grouper survival rate is very low under artificial conditions (<40%)
Major problems: Nodavirus and iridovirus oubreaks



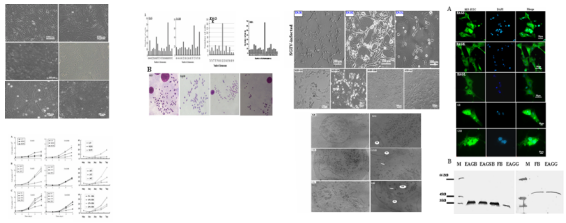
Nervous Necrosis Virus



Iridovirus

3

(I) 9 New fish cell lines susceptible to viruses established: 8 from grouper,1 from flounder



Provide cell culture platform for marine fish virus isolation, pathogenesis and vaccine development

Qin et al. J. Virol Meth, 2006.
Huang et al., Aquacultue, 2009; 2010.
Ouyang et al., J. Fish Biol., 2010.
Gong et al., J. Fish Dis., 2010

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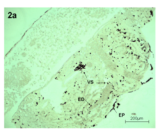
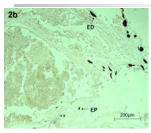
(II) A novel pathogenic iridovirus isolated from diseased grouper in Singapore, named as Singapore grouper iridovirus (SGIV)

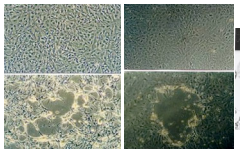
Diseased fish



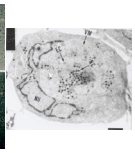
Virus infected kidney



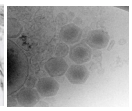
Virus infected spleen



Virus infected grouper and seabass cell cultures

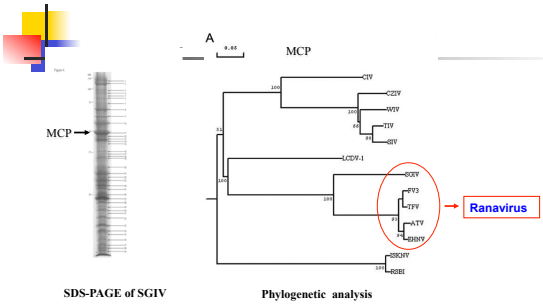


TEM of infected cell



Purifed virus particles

5



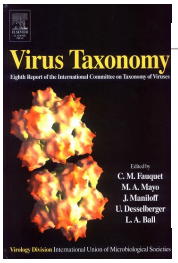
SDS-PAGE of SGIV

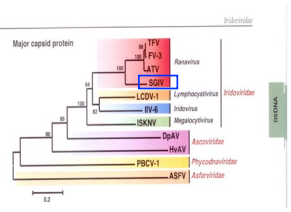
Phylogenetic analysis

Qin et al. Dis. Aquat Org., 2003. 53: 1-9.
Qin et al. J. Virol Meth., 2004. 117: 123-128.

6

SGIV has been listed as a tentative new species of genus *ranavirus*, family *iridoviridae*





Chinchar et al. 2005. In Virus Taxonomy, p161.

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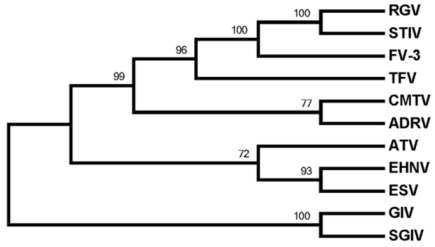

The evolutionary relationships among the 11 fully sequenced ranaviruses , based on aligned deduced amino acid sequences of 26 conserved iridovirus genes (Jancovich et al., 2015).

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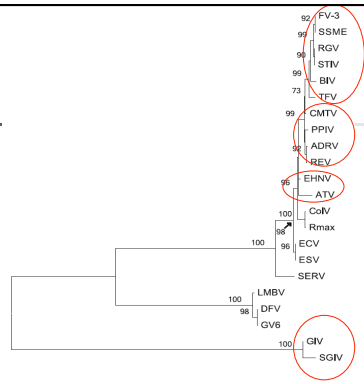

The evolutionary relationships among the 22 ranaviruses , based on full length nucleotide (nt) sequences of major capsid protein (MCP) genes (Jancovich et al., 2015). Ranaviruses could be classified into 4 groups, and group of SGIV & GIV is distinctively different from other 3 groups.

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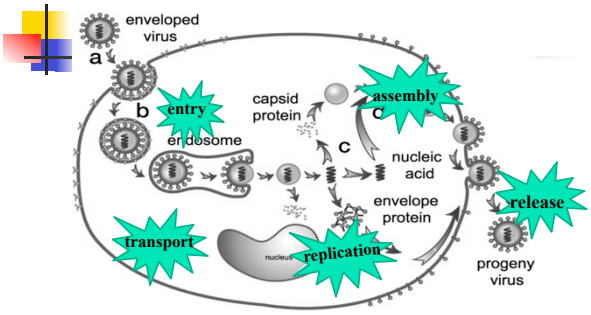
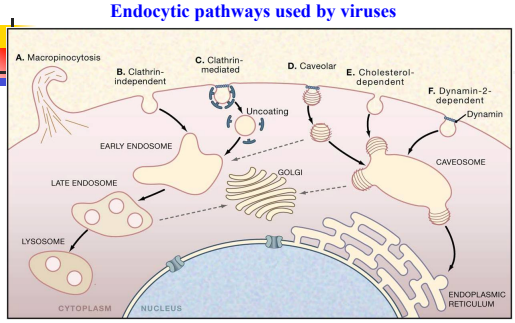
(III) The process of SGIV infection and replication



Key events during infection（take enveloped virus as an example）

Gao et al, PNAS, 2005

Endocytic pathways used by viruses



Marsh et al, Cell, 2006

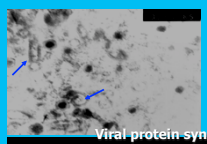
Virus entry into host cells is critically important for initiating infections and is usually recognized as an ideal target for the design of antiviral strategies.

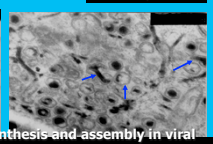
SGIV infection and replication under TEM

Virus entry

Disassembly

immature nucleocapsides





Viral protein synthesis and assembly in viral matrix

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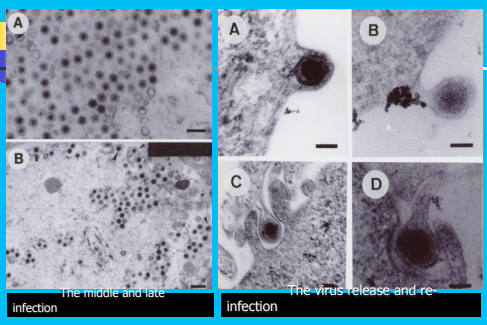

Qin et al. J. Virol Meth., 2001. 98: 17-24.
Qin et al. J. Virol Meth., 2002. 106: 89-96.
Qin et al. Dis. Aquat Org., 2003. 53: 1-9.

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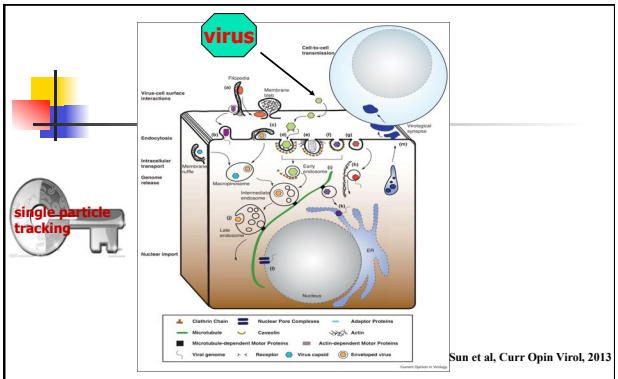
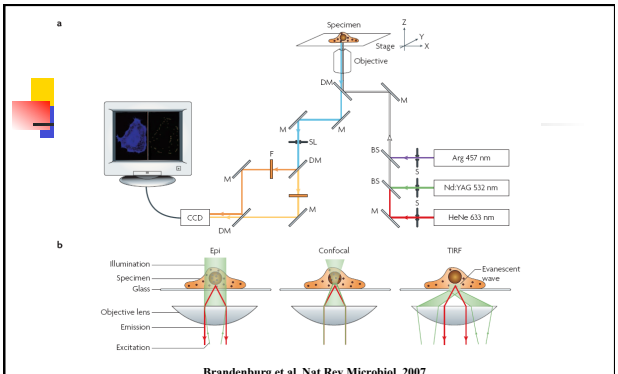


Sun et al, Curr Opin Virol, 2013

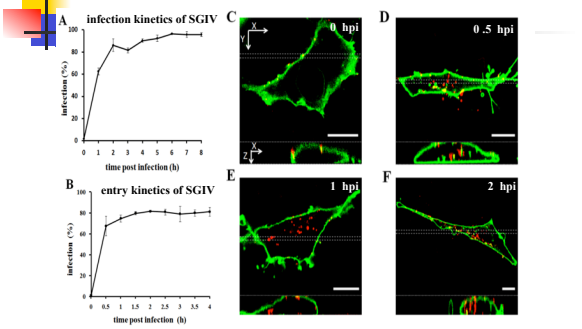
How the viruses enter into cells, how the viruses transport in cells and how the virus release genes? Such dynamic processes are in "black box", and single particle tracking is the "key" to visual the fate of viruses.



Brandenburg et al, Nat Rev Microbiol, 2007

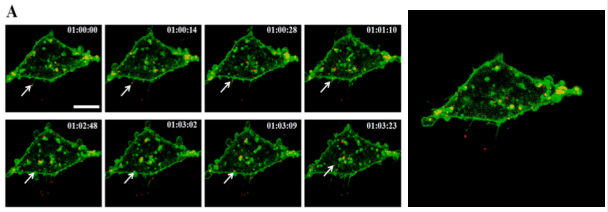
Single particle tracking is a real time imaging technique that uses fluorescence microscopy to monitor individual particles in live cells, offering us the chance to visualize the virus journey.

1）The course of SGIV entry into host cells



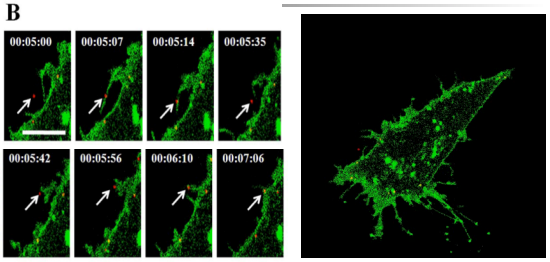
SGIV entry is relatively rapid, and complete by 2h after infection.

2）Dynamic process of SGIV entry into host cells



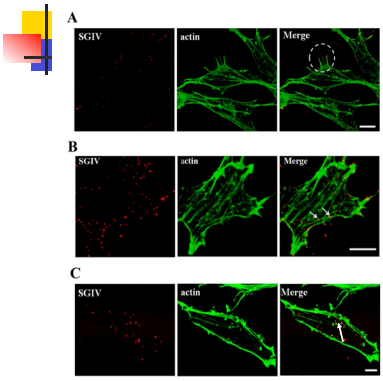
SGIV transports along filopodia-like protrusions and then enters into the cell.

2）Dynamic process of SGIV entry into host cells



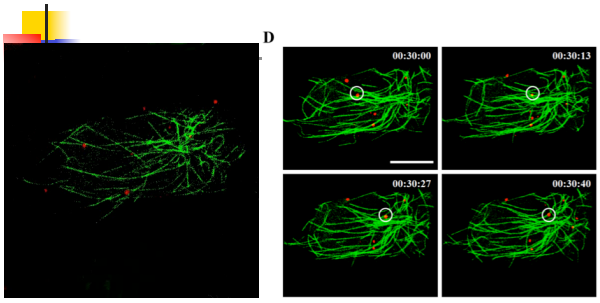
Retraction of surface protrusions serves as another means of transferring SGIV to the cell surface.

3）The motility of SGIV is dependent on the cytoskeleton



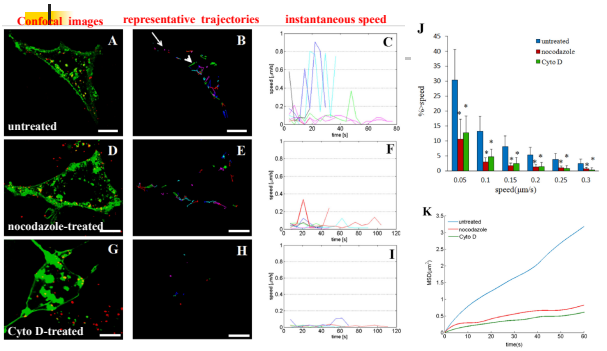
Many SGIV particles colocalize with actin.

3）The motility of SGIV is dependent on the cytoskeleton



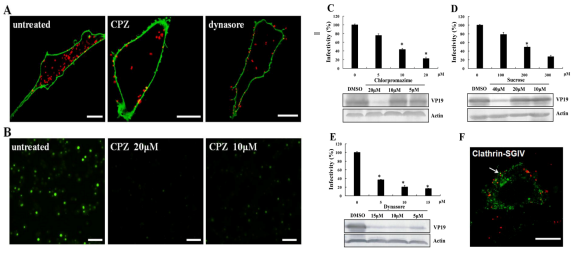
Individual SGIV particles move along microtubules.

3）The motility of SGIV is dependent on the cytoskeleton



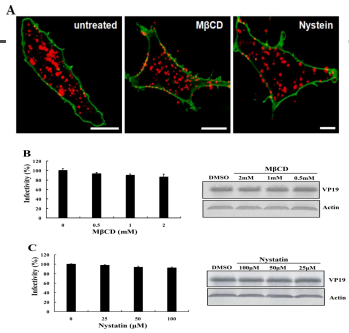
The motility of SGIV is impaired in nocodazole-treated or Cyto D-treated GS cells.

4）The endocytic pathways of SGIV entry



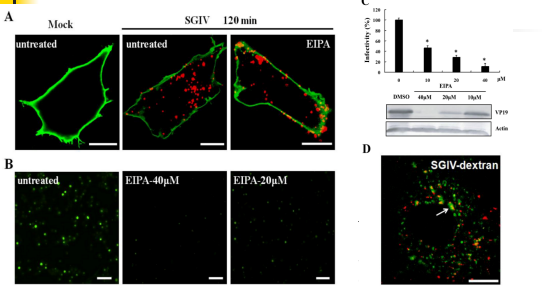
Dynamin-dependent and clathrin-mediated endocytosis is involved in SGIV entry.

4）The endocytic pathways of SGIV entry



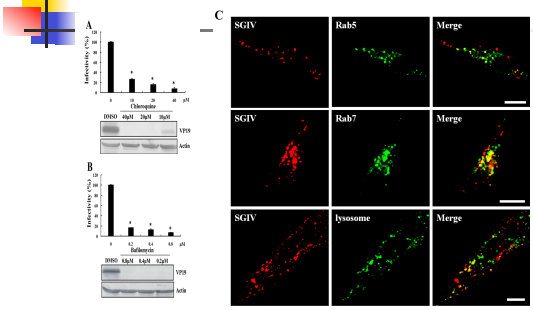
Entry of SGIV is independent of lipid raft/ caveolin-dependent endocytosis.

4）The endocytic pathways of SGIV entry



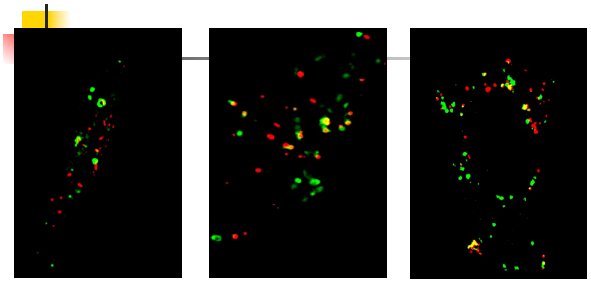
SGIV also uses micropinocytosis to enter GS cells.

4）The endocytic pathways of SGIV entry



SGIV enter host cells in pH-dependent manner.

3）the pathways of SGIV entry

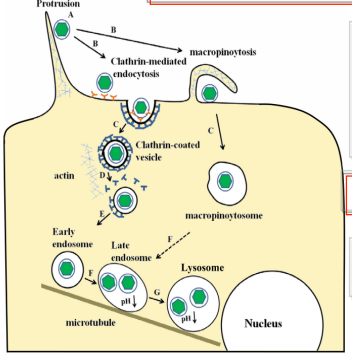


SGIV-Rab5　　SGIV-Rab7　　SGIV- Lysosome

SGIV particles transport along with early endosomes, late endosomes and lysosomes.

Conclusion

SGIV attach to and move along with filopodia



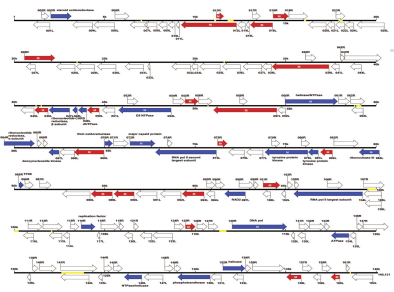
SGIV enter host cells via the clathrin-mediated endocytic pathway and macropinocytosis, but no via caveola-dependent endocytosis.

SGIV entry is pH-dependent.

The motility of SGIV depend on cytoskeleton.

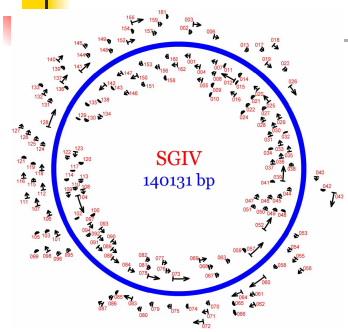
Wang et al, J Virol, 2014, 88:13047-13063

(IV) Genomics of SGIV



Organization of the SGIV genome.
The SGIV genome shown in a linear format with a full length in size of 140,131 bp. A total of 162 ORFs are indicated by their locations, orientations, and putative size. Blue arrows: ORFs with known function, red arrows: ORFs detected by RT-PCR. "M" : ORF product identified by MALDI -TOF MS/MS. Yellow lines: repetitive sequence regions. The scale is in 5 kbp.

Circular representation of SGIV genome



23 of the ORFs share high levels of identity to iridovirus proteins with known functions.

41 ORFs are homologous to other iridovirus genes, with unknown functions.

3 ORFs show weak homologies to other virus genes.

42 conserved domains, motifs, or signatures are Identified from the NCBI CD-Search database and the PROSITE database

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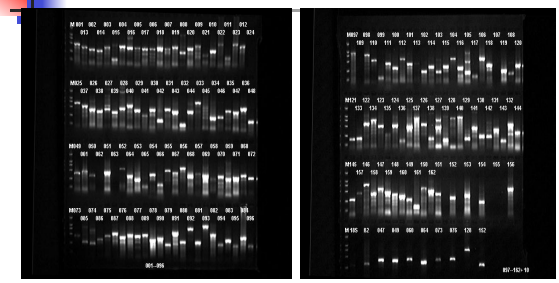
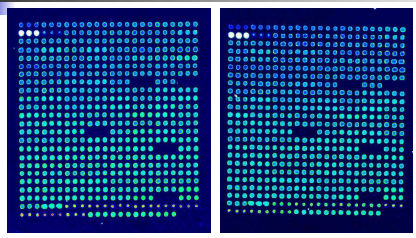
(V) Transcriptome of SGIV



PCR products amplified from all ORFs of SGIV genome for DNA microarray

30

DNA Microarrays containing SGIV all 162 ORFs (The first chip and the last chip)



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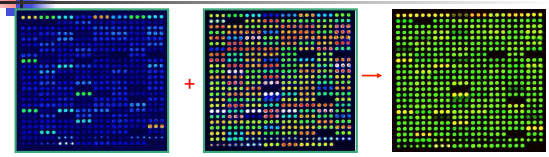
Gene transcription and expression of SGIV in virus-infected cells (post infection of 48 h)



Mock-infected control | SGIV-infected GS cells | Overlay of infected signals with control

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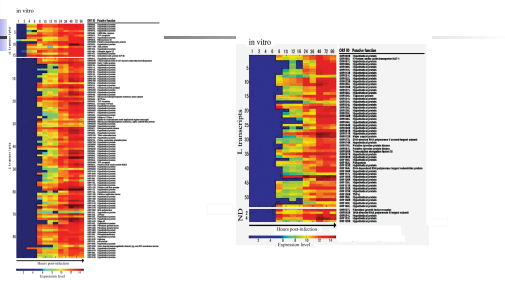
Transcription profiles of SGIV in virus-infected grouper spleen cells invitro



Columns represent separate time points, and each row indicates the transcriptional profile of a single ORF during viral infection.

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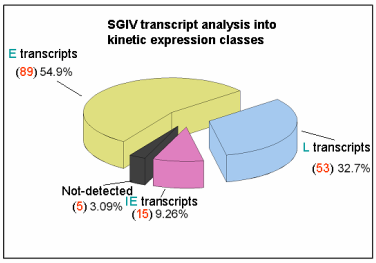
Temporal expression kinectics of SGIV transcripts



15 immediate early (IE), 89 early (E) and 53 late (L) genes were classfied.

Teng et al., 2008. Virology, 377: 39-48.

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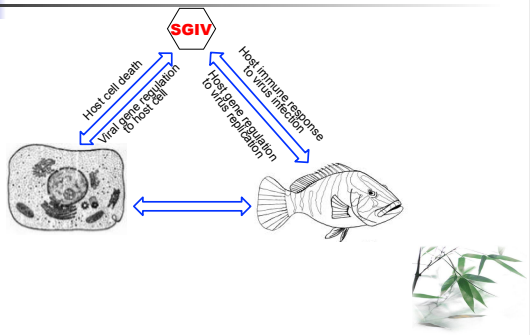
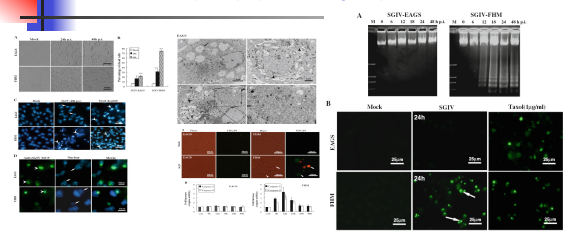
(VI) SGIV pathogenesis: interactions between virus and host



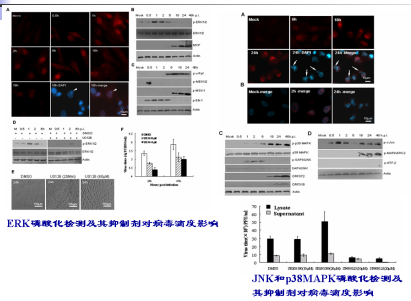
1. SGIV infection induced a new programmed cell death-parapoptosis in grouper host cells



Non-apoptotic bodies, caspse 3, 8,9 not activated, cytoplamic vaculoes, distended endoplasmic reticulum (ER), large cluster of swollen mitochondria. No DNA ladder, TUNEL negative.

(Huang et al., 2011a Apoptosis)

SGIV infection activated MAPK pathways, ERK and JNK signaling molecules involved in virus replication and virus induced cell death. Inhibition of ERK and JNK activities could delay the CPE and reduce the virus production.

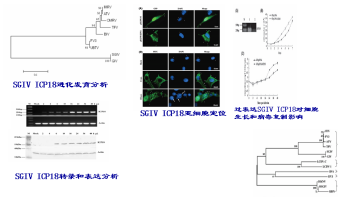


ERK磷酸化检测及其抑制剂对病毒滴度影响

JNK和p38MAPK磷酸化检测及其抑制剂对病毒滴度影响

(Huang et al., 2011b Apoptosis)

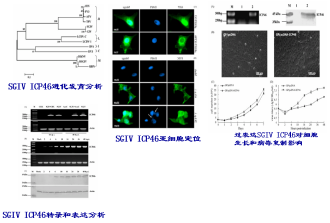
2. SGIV encoded two IE gene (ICP18, ICP46)could regulate host cell cycle , promopt cell growth, contribue virus replication.



SGIV ICP18进化关系分析

SGIV ICP18亚细胞定位

SGIV ICP18转录和表达分析



SGIV ICP46进化关系分析

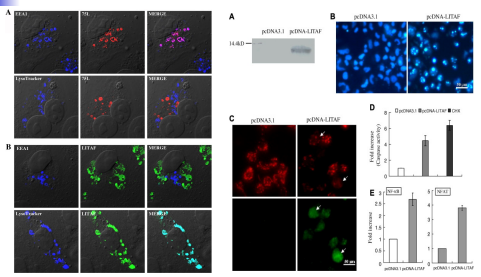
SGIV ICP46亚细胞定位

SGIV ICP46转录和表达分析

Xia et al., Arch Virol,2009;
Xia et al., Virus Res, 2010.

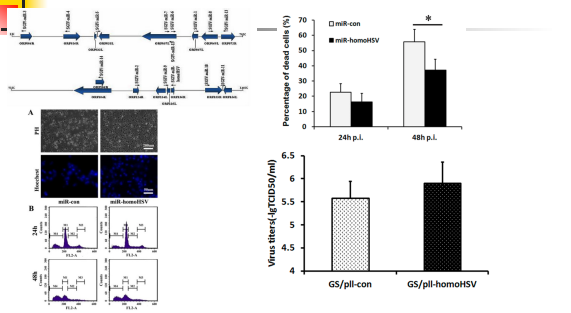
3. SGIV could encode the genes homologous to host, and manipulate host immune response (Immune evasion)

1) SGIV LITAF homologues co-localized with host LITAF , regulated expression of host LITAF, enhanced virus induced apoptosis



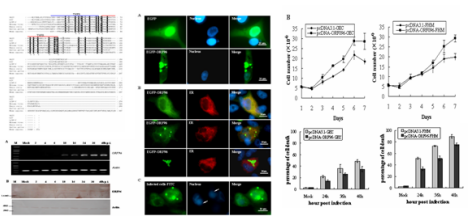
(Huang et al., 2008, BBRC)

SGIV encoded miRNA of miR-homoHSV targeted SGIV LITAF homologue , inhibited SGIV induced apoptosis and enhanced virus replication



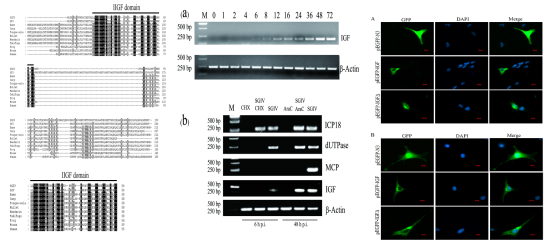
(Guo et al., 2013 Plos ONE)

2) SGIV ORF96 encodes TNFR homologue, could prompt cell growth, inhibit apoptosis, enhanced virus replication



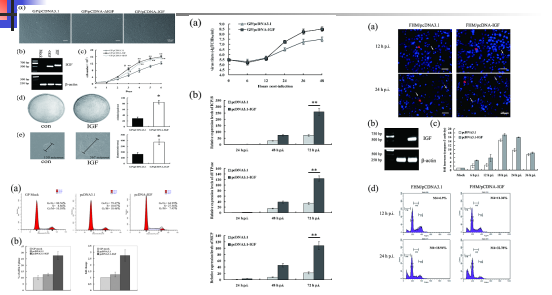
（Huang et al., 2013 Virus Res）

3) SGIV ORF062R encodes IGF homologue. SGIV IGF is an E gene, localized in cytoplasm



16

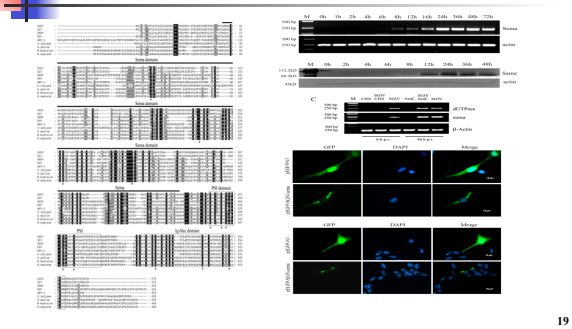
SGIV IGF could prompt cell cycle, improve host cell growth, enhance virus replication and virus induced apoptosis.



Yan et al., *J Gen Virol.* 2013 12:2759-70.

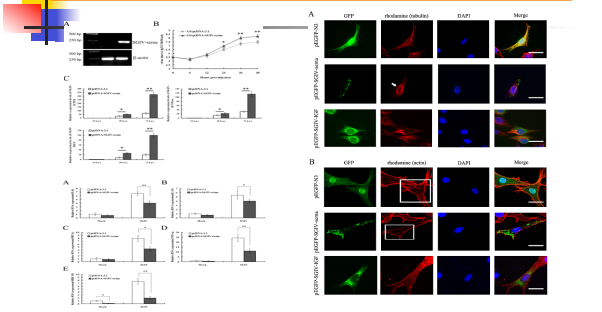
17

4) SGIV ORF155R encodes a semaphorin homologue. SGIV semaphorin is an E gene, localized in cytoplasm.



19

SGIV semaphorin could alter host cell skeleton, inhibit immune gene expression, and prompt virus replication.



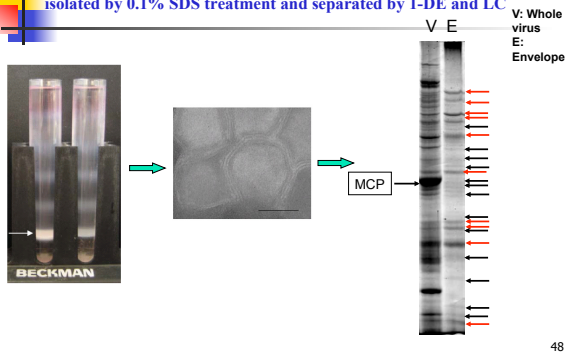
Yan et al., 2014. J Gen Virol

20

(VII) Functional proteomics of SGIV envelope proteins

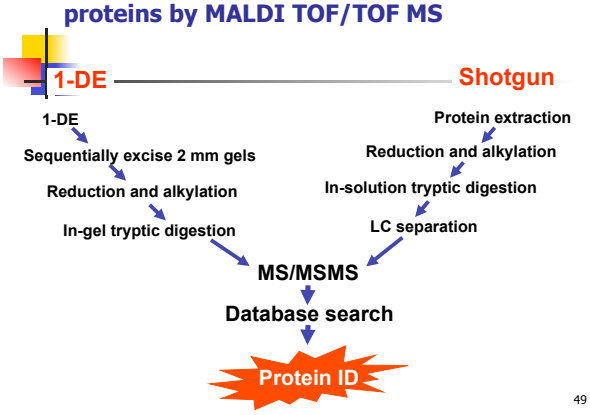
47

The complete SGIV virus particles with envelope were purified using sucrose gradient centrifugation, the viral envelope proteins were isolated by 0.1% SDS treatment and separated by 1-DE and LC



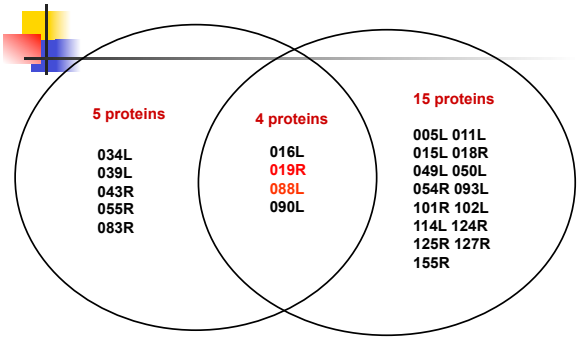
48

Proteomic Analysis of SGIV envelope proteins by MALDI TOF/TOF MS



49

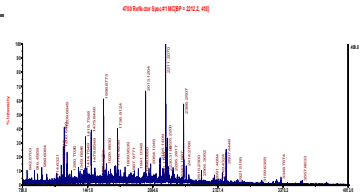
24 SGIV envelope proteins identified by both 1-DE-MALDI and LC-MALDI



Zhou et al., 2011, Proteomics

50

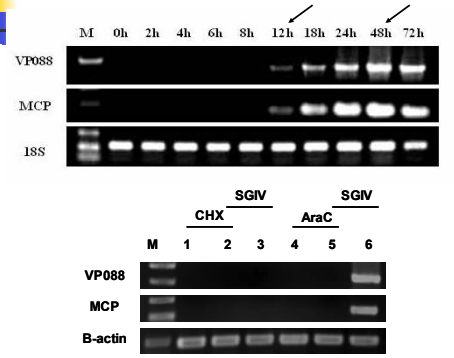
SGIV ORF088L



```
  1 MGAAQSVNGV KIVTNAYAEI MTDLAVDQDI TADQTQVFSI DNVSGDVYVE
 51 GTVMTEKLVI NLASLMKAVT NQSSQDELID NIAQQAQAAV SGLNFAQFAF
101 VTNNIDRLIT ACVKLSVDMR VSCTAKVEMT QSPSVSSVMG DVKVKDVRME
151 EPSDVISTCA LNAAITNAQL SDIVSQIKQR GDATATGLNP MAILAAVVLA
201 IIGLPLGAGF LAGRRVVGPL MMVTGMVGGG ALAMGYVPEP IKLTGPAPEP
251 DLTAVVPYAV ENGLTLKAAI SKLTGDPQYG AYYWQNYKVT GTTAVKLNQT
301 VDYYAPPNFD PVTWTASEPA QKQPSPRVFP TLPQGQGLPK ISYRLAYGTV
351 ALTQGPERGD VYLDSTTGNY YVLKDGWKLN GTIPGAIKDR PEAWGIVDPN
401 ETTALTGSER YTWVDPYTRV QGTLWYKPKD SHEWVKERQD PVPINVPLTE
451 TPSDPNVWVY KDATAKKIVG WVSAGAGAAG AGLTASAYFM PSTAVDAKAE
501 VGEAAK
```

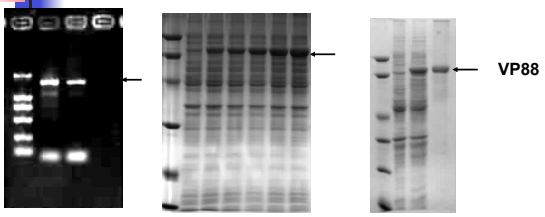
51

VP88 transcribed from 12 h p.i and drug treatment indicate VP088 is a late gene



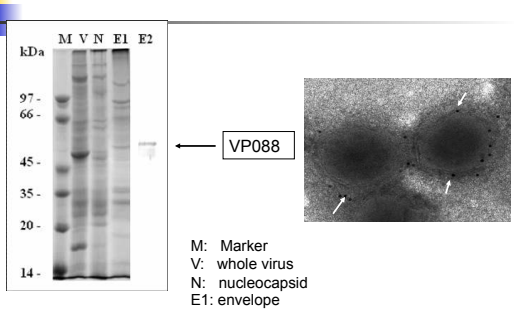
52

VP88 was cloned, expressed in E. coli and purified



53

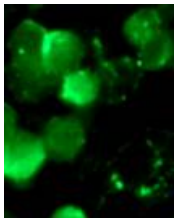
Western blot and immune-gold electron microscopy indicated that VP88 was in envelope

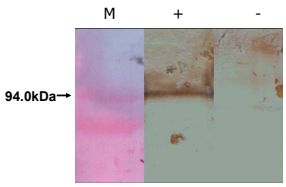


M: Marker
V: whole virus
N: nucleocapsid
E1: envelope
E2: Western blot

54

VP88 attached to the host cell membrane and reacted with a protein of 94 kDa



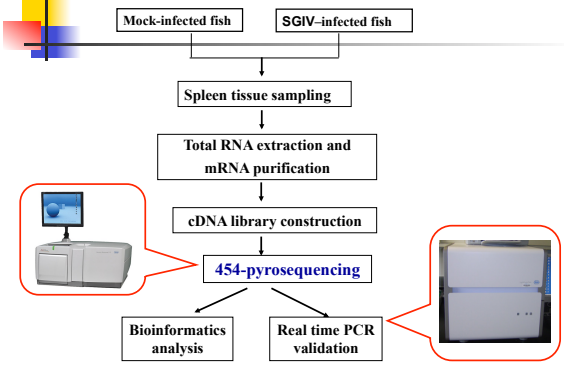


55

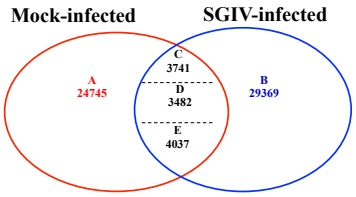
(VIII) Grouper, *Epinephelus coioides* immune responses to SGIV infection

56

1. Transcriptome analysis of grouper spleen in response to SGIV infection



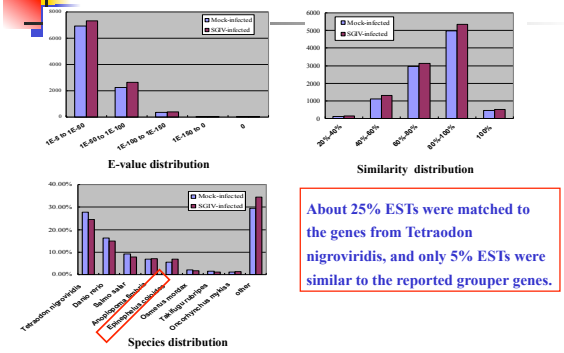
Expression of ESTs in mock- and SGIV-infected grouper spleens



(A) 24745 ESTs expressed only in control library；(B) 29369 ESTs only expressed only in infected library；(C)3741ESTs expressed higher than those in control library；(D) ESTs expressed similar both；(E) infected ESTs expressed lower than control。

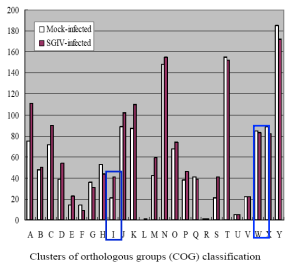
58

Characteristics of homology search of ESTs against the NCBI database



About 25% ESTs were matched to the genes from Tetraodon nigroviridis, and only 5% ESTs were similar to the reported grouper genes.

Histogram of clusters of orthologous groups (COG) classification in mock and SGIV infected libraries



A: Amino acid transport and metabolism
B: Carbohydrate transport and metabolism
C: Cell division and chromosome partitioning
D: Cell envelope biogenesis, outer membrane
E: Cell motility and secretion
F: Chromatin structure and dynamics
G: Coenzyme metabolism
H: Cytoskeleton
I: Defense mechanisms
J: DNA replication, recombination, and repair
K: Energy production and conversion
L: Extracellular structures
M: Function unknown
N: General function prediction only
O: Inorganic ion transport and metabolism
P: Intracellular trafficking and secretion
Q: Lipid metabolism
R: Nuclear structure
S: Nucleotide transport and metabolism
T: Posttranslational modification, protein turnover, chaperones
U: RNA processing and modification
V: Secondary metabolites biosynthesis, transport, and catabolism
W: Signal transduction mechanisms
X: Transcription
Y: Translation, ribosomal structure and biogenesis

60 most abundant genes in the mock-infected library

抗菌肽

Pleurocidin-like

半乳糖凝集素

Galectin-9

Chemokine receptor

趋化因子受体4

Chemokine

趋化因子4

The sixty most abundant genes in the mock-infected library

Swiss-Prot_Annotation	CDD_Annotation	GO_INFO	GENE Coverage
Hemoglobin subunit alpha	cd01040, globin,	oxygen transporter activity	19157
Hemoglobin subunit beta-2	pfam00042, globin	oxygen transporter activity	8038
Profilin-2	pfam00235, Profilin.	actin binding	3477
class II histocompatibility antigen	pfam07654, Immunoglobulin C1-set domain.	-	1699
Cytochrome c oxidase subunit 2	MTH00129, cytochrome c oxidase subunit II	cytochrome-c oxidase activity	1654
Beta-2-microglobulin	cd05770, IgC_beta2m	immune response	1529
Cytochrome c oxidase subunit 32	MTH00130, cytochrome c oxidase subunit III	cytochrome-c oxidase activity	1394
Destrin	cd00013,cofilin -like domains	cytokinesis	1340
Cytochrome c oxidase subunit 1	MTH00116, cytochrome c oxidase subunit I	cytochrome-c oxidase activity	1298
Ferritin	cd01056, eukaryotic ferritins.	ferroxidase activity	1258
40S ribosomal protein S18	PTZ00134, 40S ribosomal protein S18	translation	1138
40S ribosomal protein S24	PTZ00071, 40S ribosomal protein S24	translation	1073
Cysteine-rich protein 1	pfam00412, LIM domain.	zinc ion binding	1029
40S ribosomal protein S7	pfam01251, Ribosomal protein S7e.	translation	885
Actin	PTZ00281, actin	cytoskeleton;	883
Pleurocidin-like peptide	pfam08107, Antimicrobial12	defense response to bacterium;	836
Keratin	pfam00038, Filament	intermediate filament	828
Cytochrome b	MTH00131, cytochrome b	mitochondrion	801
Ig heavy chain Mem5	cd04981, Ig heavy chain (H),	antigen binding	682
Nucleolar GTP-binding protein 1	pfam08155, NOGCT domain	-	608
Myosin regulatory light chain 2	COG5126, Ca2+-binding protein	myosin complex;	578
60S ribosomal protein L6	pfam01159, Ribosomal protein L6e.	translation	576
Mannan-binding lectin serine protease 2	smart00042, CUB Domain	-	538
interferon-inducible lysosomal thiol reductase	pfam03227, GILT	-	465
Galectin-9	pfam00337, Galactoside-binding lectin	sugar binding;	431
Ig lambda-6 chain C region	cd07699, Immunoglobulin Constant domain.	antigen binding;	422
Saposin-C	smart00741, SapB Domains.	mitochondrion	412
Hemoglobin subunit beta-2	cd00033, CCP	oxygen transporter activity	397
Thioredoxin	pfam00085, Thioredoxin	cell redox homeostasis	385
Proteasome subunit alpha type-3	cd03751, proteasome_alpha_type_3	proteasome complex	364
Elongation factor 1	PTZ00336, elongation factor 1-alpha	nucleotide binding	323
C-X-C chemokine receptor type 4	pfam00001, 7 transmembrane receptor	signal transducer activity	316
Cystatin	smart00043, Cystatin-like domain.	endopeptidase inhibitor activity	310
40S ribosomal protein S23	PTZ00067, 40S ribosomal S23	translation	309
60S ribosomal protein L9	PTZ00027, 60S ribosomal protein L6	translation	304
Thioredoxin peroxidase	PTZ00253, tryparedoxin peroxidase	peroxidase activity	285
Proteasome subunit beta type-8	cd03761, proteasome_beta_type_5	proteasome complex	285
GTP-binding nuclear protein	smart00176, RAN	signal transduction	284
Cold shock domain-containing protein	smart00357, Cold shock protein domain.	nucleic acid binding	251
Plastin-1	smart00033, Calponin homology domain	actin binding	244
Replication protein A	cd04478, RPA2_DBD_D	DNA replication;	226
Proteasome activator complex subunit 2	pfam02252, Proteasome activator pa28 beta	proteasome complex	209
Receptor expression-enhancing protein 5	pfam03134, TB2_DP1_HVA22	protein binding	202
Proteasome subunit beta type-2	cd03758, proteasome_beta_type_2	proteasome complex	193
Nucleolar protein 16	pfam09420, Ribosome biogenesis protein	nucleolus;	193
40S ribosomal protein S19	pfam01090, Ribosomal protein S19e.	constituent of ribosome	188
Calmodulin	PTZ00184, calmodulin	calcium ion binding	186
Regulator of G-protein signaling 8	pfam00615, RGS	signal transducer activity;	180
Voltage-dependent anion-selective channel protein	cd07303, Porin3,	apoptosis	178
Proteasome subunit alpha type-2	cd03750, proteasome_alpha_type_2	proteasome complex	176
Ran-specific GTPase-activating protein	pfam00638, RanBP1 domain.	-	164
C-reactive protein	smart00159, PTX	biological_process	157
Transcription initiation factor TFIID	pfam02269, TFIID-18kDa,	regulation of transcription;	155
Ubiquitin	cd01803, Ubiquitin	protein binding	151
C-C motif chemokine 4	pfam00048, IL8,	cytokine activity	147
Receptor-type tyrosine-protein phosphatase C	smart00194, Protein tyrosine phosphatase	-	141
Cyclin-G1	smart00385, CYCLIN domain	cell cycle	130
Cytochrome c oxidase subunit 6A	cd00925, Cytochrome c oxidase subunit Via	cytochrome-c oxidase activity	128
Thioredoxin	pfam00085, Thioredoxin	cell redox homeostasis	114
Regulator of G-protein signaling 16	pfam00615, RGS	signal transducer activity	112

60 most abundant genes in the SGIV-infected library

MHC II

II型MHC

热休克蛋白90

HSP 90

I型MHC

MHC I

G型溶菌酶

Lysozyme g

泛素

Ubiquitin

趋化因子18

Chemokine 18

The sixty most abundant genes in the SGIV-infected library

Gene name	CDD_Annotation	GO_information	GENE Coverage
Ig heavy chain C region	cd00098,Immunoglobulin Constant domain	antigen binding	3999
Cytochrome c oxidase subunit 2	MTH00129, cytochrome c oxidase subunit II	cytochrome-c oxidase activity	2796
Cytochrome c oxidase subunit 3	MTH00130, cytochrome c oxidase subunit III	cytochrome-c oxidase activity	2505
Profilin-2	pfam00235, Profilin	actin binding	2356
class II histocompatibility antigen,	pfam07654, Immunoglobulin C1-set domain	-	2182
Cytochrome c oxidase subunit 1	MTH00116, cytochrome c oxidase subunit I	cytochrome-c oxidase activity	2047
Translationally-controlled tumor protein	pfam00838,TCTP	calcium ion binding	1830
Keratin	pfam00038, Intermediate filament protein	structural molecule activity	1641
Nascent polypeptide-associated complex	pfam01849, NAC domain.	transport	1494
Beta-2-microglobulin	cd05770, IgC_beta2m	MHC class I protein complex;	1383
Cytochrome b	MTH00131, cytochrome b	transport	1272
40S ribosomal protein S18	PTZ00134, 40S ribosomal protein S18	nucleic acid binding	1144
40S ribosomal protein S7	pfam01251, Ribosomal protein S7e.	constituent of ribosome	1058
Destrin	cd00013, cofilin -like domains	cytokinesis	1047
Cysteine-rich protein 1	pfam00412, LIM domain.	zinc ion binding	912
V-type proton ATPase subunit	pfam05493, ATP synthase subunit H.	transport	882
Actin	PTZ00281, actin	nucleotide binding	848
Pleurocidin-like peptide	pfam08107, Antimicrobial12	response to bacterium;	817
Cysteine dioxygenase type 1	pfam05995, Cysteine dioxygenase type I	iron ion binding	780
cysteine-rich type 1 protein	smart00202, Scavenger receptor Cys-rich	scavenger receptor activity	762
Eukaryotic translation initiation factor 4	pfam02020, eIF4-gamma	-	698
60S ribosomal protein L10	PTZ00173, 60S ribosomal protein L10	constituent of ribosome	689
60S ribosomal protein L19	cd01417, Ribosomal protein L19e	constituent of ribosome	598
Nucleoside diphosphate kinase	PTZ00093, nucleoside diphosphate kinase	nucleotide binding	592
Thioredoxin	pfam00085, Thioredoxin	transport	583
Palmitoyl-protein thioesterase 1	pfam02089,Palmitoyl protein thioesterase	-	555
Heat shock cognate protein HSP 90-beta	pfam00183, Hsp90 protein.	nucleotide binding	495
Nicotinamide riboside kinase 2	cd02024, Nicotinamide riboside kinase	-	487
Legumain	pfam01650, Peptidase_C13	endopeptidase activity	483
Galectin-9	pfam00337, Galactoside-binding lectin.	sugar binding;	423
60S ribosomal protein L9	PTZ00027, 60S ribosomal protein L9	constituent of ribosome	409
Class I histocompatibility antigen,	pfam00129, MHC_I	-	408
Proteasome subunit alpha type-3	cd03751, proteasome_alpha_type_3,	proteasome complex	405
Proteasome subunit beta type-8	cd03761, proteasome_beta_type_5	proteasome complex	367
Elongation factor 1-alpha	PTZ00336, elongation factor 1-alpha	nucleotide binding	363
Cathepsin B	cd02620, Cathepsin B group	endopeptidase activity	361
Ig lambda-6 chain C region	cd07699, Immunoglobulin Constant domain	antigen binding;	349
Myosin light polypeptide 6	PTZ00184, calmodulin;	motor activity	338
Lysozyme g	cd01021, Goose Egg White Lysozyme domain	lysozyme activity	322
Proteasome subunit alpha type-7	cd03755, proteasome_alpha_type_7	proteasome complex	316
interferon-inducible lysosomal thiol reductase	pfam03227, GILT	-	307
NADH-ubiquinone oxidoreductase chain 4	MTH00127, NADH dehydrogenase subunit 4	mitochondrion	300
Cystatin	smart00043, Cystatin-like domain.	endopeptidase inhibitor activity	298
Nattectin	smart00034, C-type lectin (CTL)	binding	256
Ubiquitin	cd01803, Ubiquitin,	protein binding	242
Fucolectin	smart00607, Pentaxrin-1 Domain.	complement activation	226
Calmodulin	PTZ00184, calmodulin;	receptor binding	225
Eukaryotic translation initiation factor 3	pfam09440, eIF3 subunit 6	translation initiation factor activity	225
Ubiquitin	cd01803, Ubiquitin,	transcription regulator activity	197
Fucolectin-4	smart00607,eel-Fucolectin Domain.	lectin pathway	183
C-reactive protein	smart00159, C-reactive protein	protein binding	168
Proteasome subunit beta type-3	cd03759, proteasome_beta_type_3	proteasome complex	158
Leukocyte elastase inhibitor	cd02058, Plasminogen Activator Inhibitor-2	endopeptidase inhibitor activity	151
C-X-C chemokine receptor type 4	pfam00001,7 transmembrane receptor	signal transducer activity	142
Cyclin-G1	smart00385, CYCLIN	cell cycle	134
Calmodulin	PTZ00184, calmodulin	calcium ion binding	133
C-C motif chemokine 18	smart00199, small cytokine C-X-C	cytokine activity	130
Natural killer cell protease 1	smart00020, Trypsin-like serine protease	serine-type endopeptidase activity	121

755 genes were significantly up-regulated in response to SGIV infection

半胱氨酸蛋白酶 抑制剂

CC 趋化因子 18

G型溶菌酶

白细胞介素8

泛素连接酶 E2

Table 3

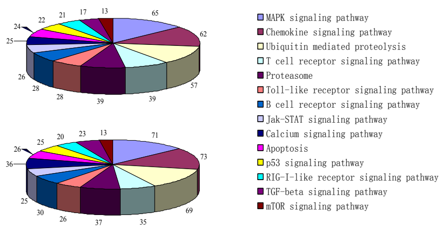
Gene name	putative feature/function	species	expression in normal	Expression in infection	Fisher p_value
Profilin-2		Rattus norvegicus	305	29051	0
60S ribosomal protein		Homo sapiens	251	23569	0
Eotaxin		Mus musculus	3169	17689	0
Granulins		Rattus norvegicus	1141	15720	0
Leukocyte cell-derived chemotaxin-2		Mus musculus	4809	10637	0
Thioredoxin		Ictalurus punctatus	542	7343	0
Saposin-C		Cavia porcellus	43	5116	0
Cystatin-B 1		Macaca fuscata	92	4388	0
Perforin-1		Mus musculus	105	4097	0
Scavenger receptor cysteine-rich type 1 protein		Canis familiaris	911	3958	0
Eukaryotic translation initiation factor 4 gamma 2		Gallus gallus	573	3829	0
Gamma-glutamyl hydrolase		Homo sapiens	133	2638	0
Legumain		Bos taurus	445	2572	0
Proteasome subunit alpha type-7		Carassius auratus	35	2452	0
Glyceraldehyde 3-phosphate dehydrogenase,		Danio rerio	103	1983	0
Transcription factor BTF3		Mus musculus	83	1770	0
Src-like-adapter		Mus musculus	84	1371	2.02E-303
Keratin		Gallus gallus	182	1586	5.86E-283
Galectin-3-binding protein B		Danio rerio	450	2187	8.23E-277
SUMO-conjugating enzyme		Xenopus tropicalis	137	1307	8.94E-243
C-C motif chemokine 18		Macaca mulatta	282	1667	3.00E-242
Galectin-3-binding protein A		Danio rerio	371	1747	3.37E-216
Hemoglobin subunit beta-1		Pseudaphritis urvillii	241	1383	1.38E-197
Keratin		Oncorhynchus mykiss	1661	3701	7.37E-180
Eukaryotic translation initiation factor 3		Bos taurus	67	844	4.27E-174
Cytochrome b		Tropheus moorii	6544	9945	1.29E-163
Natural killer cell protease 1		Rattus norvegicus	227	1138	1.95E-148
T-complex protein 1		Gallus gallus	50	669	6.61E-141
RNA-binding protein 5		Xenopus tropicalis	45	605	7.97E-128
Cathepsin H		Sus scrofa	391	1347	3.79E-125
Beta-2-glycoprotein 1		Bos taurus	433	1389	7.97E-119
Dipeptidyl peptidase 1		Bos taurus	55	586	1.36E-114
Proto-oncogene vav		Mus musculus	98	693	8.51E-113
Proteasome subunit alpha type-4		Homo sapiens	684	1761	2.56E-111
Actin-related protein 2/3 complex subunit 5		Mus musculus	90	656	1.35E-108
Proteasome subunit beta type-6-B like		Salmo salar	469	1361	8.49E-103
RING-box protein 2		Mus musculus	42	497	3.76E-101
NADH-ubiquinone oxidoreductase chain 1		Carassius auratus	330	1102	2.68E-99
Phospholipid hydroperoxide glutathione peroxidase		Sus scrofa	590	1527	1.66E-97
Retinol-binding protein 1		Bos taurus	164	767	1.80E-95
NudC domain-containing protein 2		Rattus norvegicus	81	576	3.36E-94
Lysozyme g		Epinephelus coioides	164	742	5.02E-90
Voltage-gated hydrogen channel 1		Danio rerio	217	742	4.09E-69
Ubiquitin-like modifier-activating enzyme 5		Xenopus laevis	179	670	8.17E-69
LRR and PYD domains-containing protein 1		Homo sapiens	205	714	6.02E-68
Cytochrome c oxidase subunit 6B1		Tarsius syrichta	60	395	2.33E-62
Ig lambda chain		Homo sapiens	31	315	2.99E-61
Interleukin-8		Equus caballus	155	572	4.14E-58
Fibroleukin		Homo sapiens	611	1281	3.75E-56
Rho GDP-dissociation inhibitor 1		Macaca fascicularis	325	844	4.69E-55
Ependymin		Notemigonus crysoleucas	70	386	5.52E-55
Fucolectin-4		Anguilla japonica	1519	2434	1.29E-50
Complement factor H		Homo sapiens	131	459	1.88E-44
Eukaryotic initiation factor 4A-III		Xenopus tropicalis	56	282	4.32E-38
Cysteine and glycine-rich protein 2		Mus musculus	61	290	1.68E-37
Proliferating cell nuclear antigen		Haplochromis burtoni	237	575	3.64E-34
Secernin-3		Danio rerio	64	277	2.42E-33
Ubiquitin-conjugating enzyme E2		Mus musculus	74	286	4.09E-31
Heat shock cognate 71 kDa protein		Oryzias latipes	25	181	8.31E-31

695 genes were significantly down-regulated in response to SGIV infection

Table 4 Unique genes with decreased expression in spleen after SGIV infection

	swissprot_annotation	putative feature/function	species	Expression in control	Expression in infection	Fisher p_value
II型MHC	H-2 class II histocompatibility antigen		Mus musculus	11365	1044	0
	Glutathione peroxidase 1		Bos taurus	3469	509	0
	Endothelial differentiation-related factor 1		Xenopus laevis	5830	172	0
	Inositol-3-phosphate synthase 1-A		Xenopus laevis	1618	148	0
	Palmitoyl-protein thioesterase 1		Mus musculus	6570	92	0
	Proteasome subunit beta type-2		Bos taurus	2244	84	0
	Cytochrome c oxidase subunit 1		Gadus morhua	7690	66	0
	Myosin light polypeptide 6		Rattus norvegicus	1733	65	0
	Eukaryotic translation initiation factor 3 subunit		Danio rerio	1765	60	0
R 干扰素诱导的溶酶体巯基还原酶	Gamma-interferon-inducible lysosomal thiol reductase		Homo sapiens	2962	55	0
	Mid1-interacting protein 1-like		Danio rerio	3667	54	0
	Elongation factor 1-gamma		Carassius auratus	1821	51	0
	Plastin-2		Danio rerio	1777	51	0
	ribosomal protein S8		Rattus norvegicus	1737	48	0
	Stefin-C		Bos taurus	1991	46	0
	Complement C1q subcomponent subunit A		Bos taurus	1511	43	0
	ribosomal protein L22		Xenopus tropicalis	2286	33	0
	ribosomal protein L18		Oreochromis niloticus	1598	29	0
	Nucleolar protein 16		Tetraodon nigroviridis	1949	377	9.53E-251
	Transcription initiation factor TFIID		Pongo abelii	2123	469	6.58E-246
	Transforming protein RhoA		Rattus norvegicus	1258	202	4.46E-184
	Peroxiredoxin		Cynops pyrrhogaster	2563	982	7.87E-157
	Myosin regulatory light chain 2		Gallus gallus	5634	3124	2.64E-154
热休克蛋白70	Heat shock 70 kDa protein		Canis familiaris	612	31	7.54E-140
	Ras-related C3 botulinum toxin substrate 2		Bos taurus	928	167	1.56E-126
	SH3 domain-containing protein		Mus musculus	609	48	1.33E-123
	Rab GDP dissociation inhibitor beta		Rattus norvegicus	602	48	9.70E-122
	Cystatin-A5		Sus scrofa	2339	994	4.94E-120
	Radixin		Mus musculus	675	77	8.53E-119
干扰素诱导GTP酶	Interferon-inducible GTPase 1		Homo sapiens	805	181	4.16E-93
	C-X-C chemokine receptor type 4		Papio anubis	1929	851	1.51E-92
	AIG2-like domain-containing protein		Danio rerio	617	128	1.59E-76
	Major histocompatibility complex class I-related gene		Mus musculus	1058	366	1.83E-76
	Thioredoxin-dependent peroxide reductase,		Homo sapiens	769	209	1.20E-74
	Death-associated protein-like 1-B		Xenopus laevis	1417	638	9.05E-66
	RING finger protein 10		Mus musculus	277	24	3.52E-55
	Mannan-binding lectin serine protease 2		Mus musculus	2177	1249	6.20E-55
	GTP-binding nuclear protein		Salmo salar	3669	2432	9.56E-54
	Regulator of G-protein signaling 8		Danio rerio	1206	639	6.61E-39
	Matrix metalloproteinase-9		Homo sapiens	199	21	2.33E-37
	Bleomycin hydrolase		Gallus gallus	418	125	2.50E-37
	Src kinase-associated phosphoprotein 2		Takifugu rubripes	257	47	8.27E-36
干扰素诱导蛋白44	Interferon-induced protein 44-like		Mus musculus	352	94	1.44E-35
	Protein disulfide-isomerase A4		Rattus norvegicus	191	21	2.56E-35
	Macrophage mannose receptor 1		Homo sapiens	257	49	8.14E-35
	Prothymosin alpha-B		Danio rerio	186	21	4.75E-34
	Ubiquitin-conjugating enzyme E2		Drosophila melanogaster	344	97	6.36E-33
	Myosin-8		Canis familiaris	1023	547	1.60E-32
	cAMP-responsive element-binding protein		Danio rerio	684	312	8.58E-32
	Programmed cell death protein 10		Mus musculus	383	123	1.42E-31
	Peroxisomal membrane protein 2		Bos taurus	612	267	3.46E-31
	E3 ubiquitin-protein ligase		Homo sapiens	220	41	2.02E-30
	Myoferlin		Xenopus tropicalis	262	62	4.71E-30
	Ras-related protein Rab7		Gossypium hirsutum	223	50	6.44E-27
	Dynactin subunit 6		Homo sapiens	242	61	2.40E-26
	protease regulatory subunit 4		Rattus norvegicus	229	55	4.43E-26
干扰素调节因子1	Interferon regulatory factor 1		Gallus gallus	446	183	1.06E-25
	Hephaestin-like protein 1		Mus musculus	276	88	2.05E-23
	Galectin-8		Homo sapiens	158	29	1.39E-22
	Apoptosis-associated speck-like protein		Danio rerio	497	233	2.41E-22

KEGG analysis of ESTs involved in signaling pathways

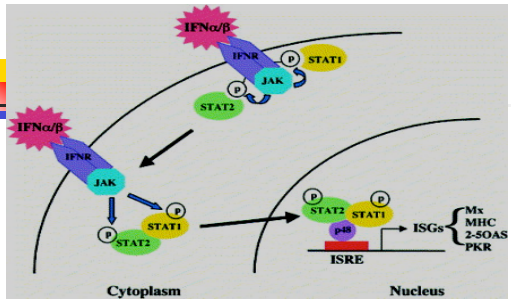


Huang YH et al. BMC Genomics, 2011.

65

2、Molecular cloning, expression and functional analysis of ISG15 from the grouper transcriptome

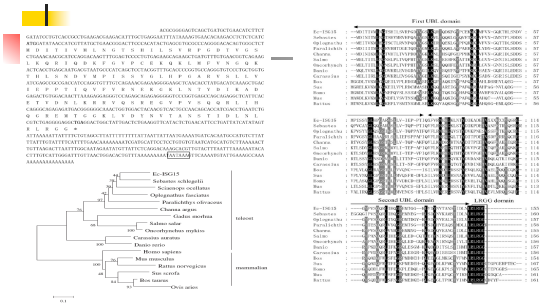
Huang XH et al. Fish & Shellfish Immunology, 2013.



Taylor DR et al., Microbes and Infection, 2000

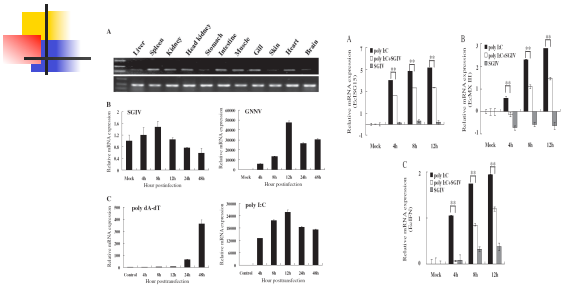
IFN-α signaling. The binding of IFN to its receptor (IFNR) activates the Janus kinase (JAK) and induces phosphorylation of STAT1 and STAT2. The phosphorylated STATs translocate from the cytoplasm to the nucleus and complex with p48 to form ISGF3, which activates transcription of the IFN-stimulated genes (ISGs).

Cloning and Bio-informatic analysis of EcISG15



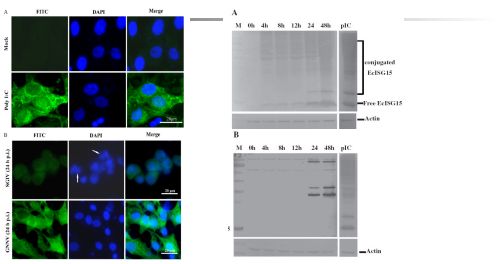
EcISG15 encoded a polypeptide of 155 amino acids and shared the highest amino acid similarity to ISG15 of *Sebastes schlegelii,* and showed different identity to other known fish ISG15 homologs.EcISG15 shared the typical characteristics of known ISG15s with two UBL domains and a c-terminal LRGG motif.

Tissue distribution and induction of EcISG 15



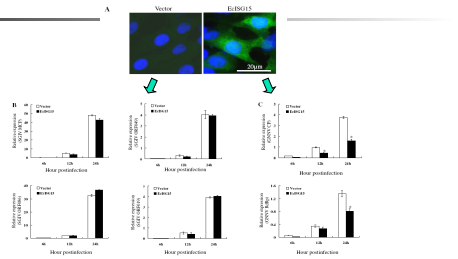
EcISG15 was dramatically induced by GNNV infection, poly I:C or poly dA-dT treatment. and no significant change of EcISG15 expression was detected in SGIV-infected cells. SGIV could prevent the expression of EcISG15, IFN and Mx induced by poly I:C, suggesting that SGIV was able to destroy the cellular interferon-mediated antiviral activity in *vitro*.

Subcellular localization and formation of EcISG15 conjugates



EcISG15 expression and ISGylation were evoked by GNNV infection or poly I:C stimulation, but not SGIV. The possibility of the absence of conjugation in SGIV infected-cells might be due to the disruption of IFN regulated response by SGIV infection in vitro.

The effects of EcISG 15 overexpression on SGIV and GNNV gene transcription

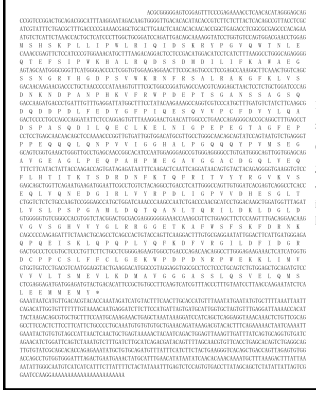


Overexpression of EcISG15 *in vitro* inhibited GNNV replication but not SGIV, suggesting that EcISG15 played the antiviral role via protein ISGylation in response to RNA virus infection.

3. Identification and functional characterization of grouper IRF3

Huang et al., 2015, Fish Shellfish Immunol

Molecular cloning of *Epinephlus coioide* IRF3 gene



EcIRF3 cDNA is of 2513 bp

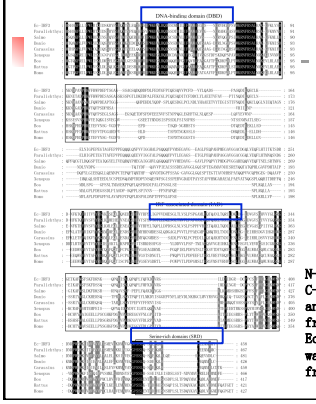
5' UTR: 230 bp

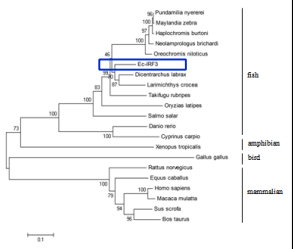
Open reading frame (ORF): 1377 bp---458 aa

3' UTR: 906 bp

The polyadenylation signal (AATAAA) and two mRNA instability motifs (ATTTTA, ATTTA) were followed by a poly(A) tail in the 30-UTR of EcIRF3 cDNA.

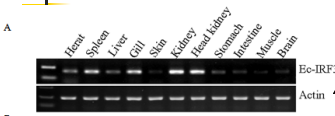
Alignment and phylogenetic analysis of EC-IRF3



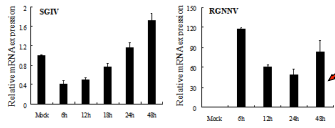


N-terminal DNA-binding domain (DBD), C-terminal IRF association domains (IAD) and serine-rich domain (SRD) were conserved from fish to mammals. EcIRF3 and other members of IRF3 subfamily was consistent with the conserved domains from lower vertebrates and mammals.

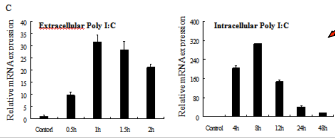
Tissue distribution and transcription pattern of Ec-IRF3



Ec-IRF3 mRNA was detected in all tissues examined, and it was expressed strongly in spleen, kidney, head kidney, gill and heart.



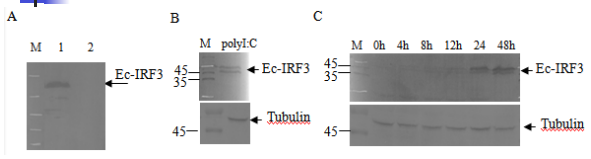
Ec-IRF3 transcript was increased significantly during RGNNV infection compared with mock-infected cells.



Ec-IRF3 transcript was significantly up-regulated upon the inductionwith extracellular or intracellular poly I:C.

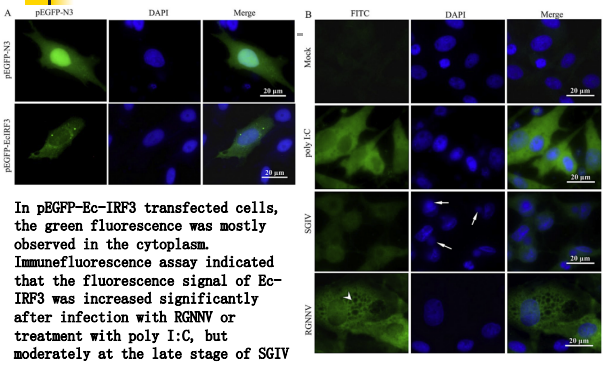
These data indicated that viral ssRNA and dsRNA could induce significant increase of Ec-IRF3 expression in grouper cells, suggesting that Ec-IRF3 might be

Expression of rEc-IRF3 and antibody preparation



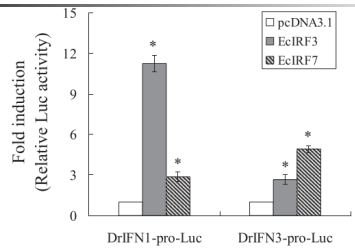
Western blotting assay showed that anti-Ec-IRF3 specially recognized not only the recombinant fused protein His-Ec-IRF3, but also the newly synthesized Ec-IRF3 proteins in poly I:C treated or RGNNV infected cells

Subcellular localization of Ec-IRF3 in grouper cells



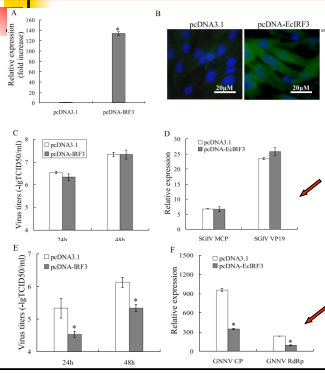
In pEGFP-Ec-IRF3 transfected cells, the green fluorescence was mostly observed in the cytoplasm. Immunefluorescence assay indicated that the fluorescence signal of Ec-IRF3 was increased significantly after infection with RGNNV or treatment with poly I:C, but moderately at the late stage of SGIV infection.

Induction of IFN promoter activity by Ec-IRF3



Reporter gene assay showed that EcIRF3 activated zebrafish type I IFN and type III IFN promoter *in vitro*. our results suggested that EcIRF3 might be involved in the regulation of IFN expression.

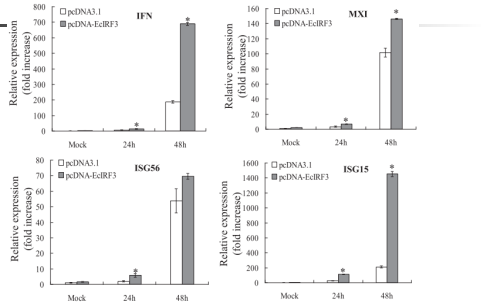
Overexpression of Ec-IRF3 inhibited RGNNV replication, but not SGIV



Ec-IRF3 did not affect the gene transcription and virus production of SGIV.

The viral gene transcription and virus production of RGNNV were significantly decreased in Ec-IRF3 overexpressing cells.

Overexpression of Ec-IRF3 increased the expression of IFN inducible genes



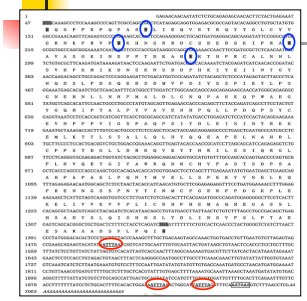
the mRNA expression levels of type I IFN and IFN-inducible genes (MxI, ISG15 and ISG56) were increased in RGNNV infected Ec-IRF3 overexpressing cells compared to empty vector transfected cells.

4. Identification and functional characterization of grouper IRF7

Cui et al., 2011, Dev Comp Immunol

1

Molecular cloning of Epinephlus coioide IRF7 gene



EcIRF7 cDNA is of 2089 bp

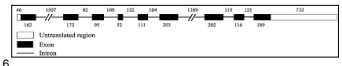
5' UTR: 46 bp

Open reading frame (ORF): 1302 bp---433 aa

3' UTR: 741 bp

Four well-spaced tryptophan residues

Three mRNA instability motifs (ATTTA)

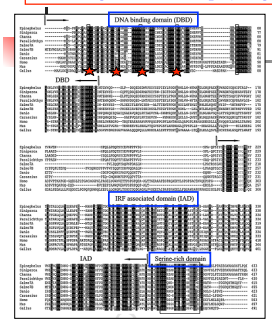


EcIRF7 genomic DNA is about 5.7 kb

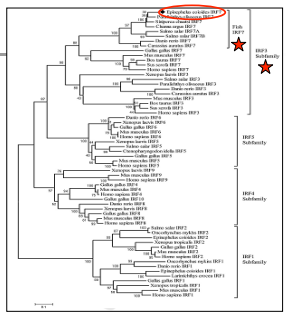
9 exons/8 introns & GT/AG rule

6

Alignment and phylogenetic analysis of EC-IRF7



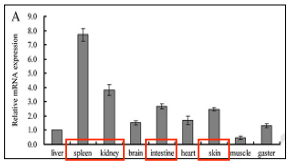
Conserved domains across species: DBD; IAD; Serine-rich domain; Tryptophan residues



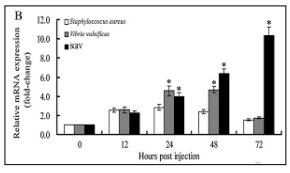
Vertebrate IRF7 subgroup, IRF3 subfamily. Closer phylogenetic distance to other fish IRF7s

7

Ec-IRF7 expression profiles in healthy and pathogens-stimulated fish



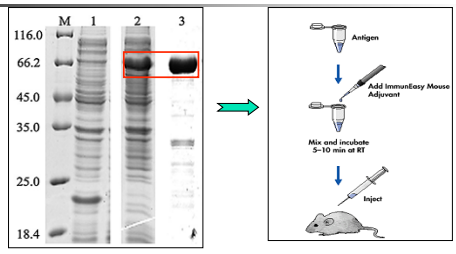
Ec-IRF7 showed strong expression in immune-related tissues (spleen, kidney, intestine et al.);



Ec-IRF7 was greatly up-regulated in spleen after stimulation with Vibrio vulnificus and Singapore grouper iridovirus (SGIV)

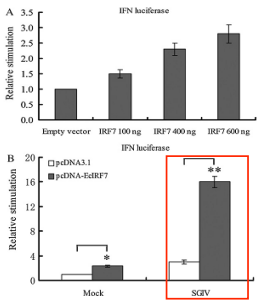
9

Expression of rEc-IRF7 and antibody preparation



8

Ec-IRF7 activates fish type I IFN promoter

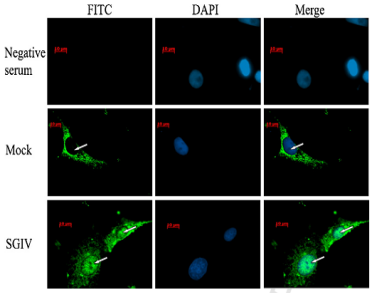


Ec−IRF7 was able to activate zebrafish IFN promoter in a dose-dependent manner;

SGIV stimulation could enhance the activation activity of Ec−IRF7 on IFN promoter

10

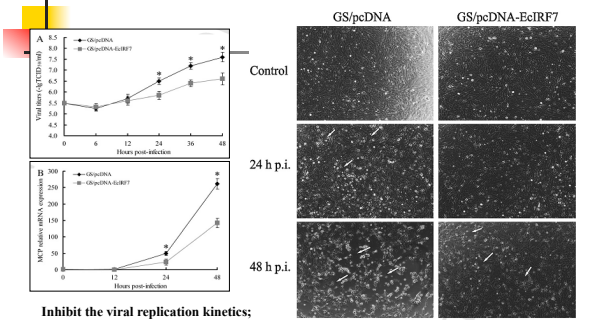
Subcellular localization of Ec-IRF7



In non-stimulated cells, Ec−IRF7 resides in the cytoplasm;
After SGIV infection, Ec−IRF7 translocated into the nucleus;
(indicating Ec−IRF7 might be phosphorylated and activated during SGIV infection)

11

Over-expressed Ec-IRF7 inhibit SGIV replication in vitro

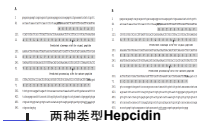


Inhibit the viral replication kinetics;

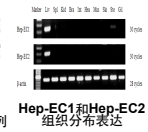
Delay the transcription kinetics of viral genes; **Delay the appearance of SGIV-induced CPE**

12

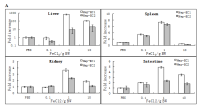
5. Grouper hepcidin (Ec-Hep)



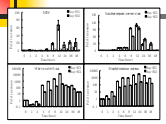
两种类型Hepcidin（Hep-EC1和Hep-EC2）的序列



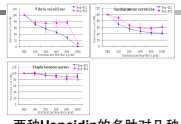
Hep-EC1和Hep-EC2组织分布表达



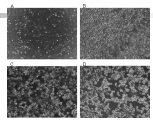
铁离子刺激后两种Hepcidin组织分布表达



病原刺激后两种Hepcidin组织分布表达



两种Hepcidin的多肽对几种菌生长的影响

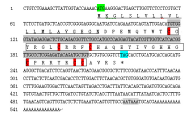


两种Hepcidin的多肽对SGIV生长的影响

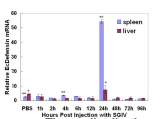
Hep1 and Hep2 highly expressed in grouper liver, pathogen or iron stimulation could up-regulate hep expression, and hep1 was much more regulated. Grouper hepcidins could inhibit bacterial and SGIV replication.

Zhou JG et al., 2010, Fish and Shellfish Immunology

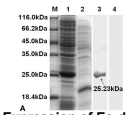
5. Grouper defesin（Ec-defesin）



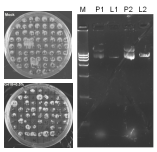
Sequence analysis



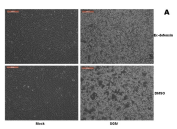
Tissue distribution cheallenged with SGIV



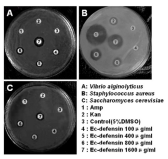
Expression of Ec-defesin in E. coli



Expression of Ec-defesin in yeast



Ec-defesin inhibited SGIV replication

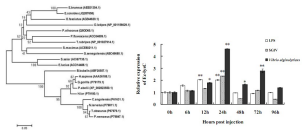


Ec-defesin inhibited micobes

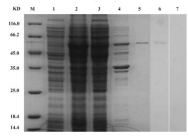
Guo et al., 2012, Fish and Shellfish Immunology

6. Grouper C-type lysozyme (Ec-lysC)

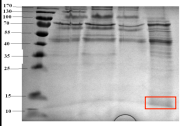
Sequence analysis



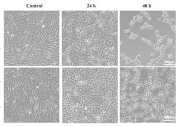
Tissue distribution chellenged with SGIV



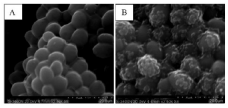
Expression in E. coli



Expression in yeast



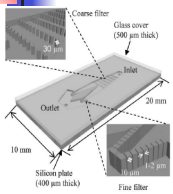
Ec-lysC inhibited SGIV replication

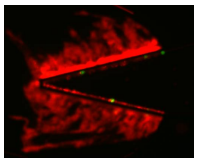


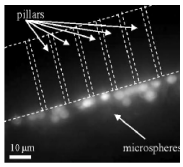
Ec-lysC could kill bacterial

Wei et al., 2012, Fish and Shellfish Immunology

VIIII Diagnostics and vaccine development







1. Using mAb against SGIV, the platform of Lab-on-chip was developed, the Lab-on-chip could rapidly detect not only SGIV-infected cells,but also free virions in water envionment.

Liu, et al., 2005. Lab on a chip.

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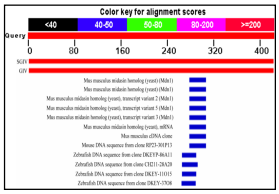
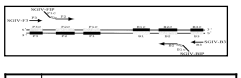
2. A LAMP specifically detect SGIV

The target gene ORF-014L specific for SGIV and GIV



Primers design



F3	GCACGAGTATACGGCCTCTG
B3	CGGCTACCAGCATCCAAT
FIP	GACTACGGGTTCGATGGCTGC-TTTT-AGACGATTGCGCATGAAACA
BIP	ACGGGAGGAAACTTGTGCTGAT-TTTT-ATGGCCCGTAGAAGTCAGT
LF	TCGCGGTTCGGGTCATCAT
LB	TGCTGGAACGTGCAAAACCG

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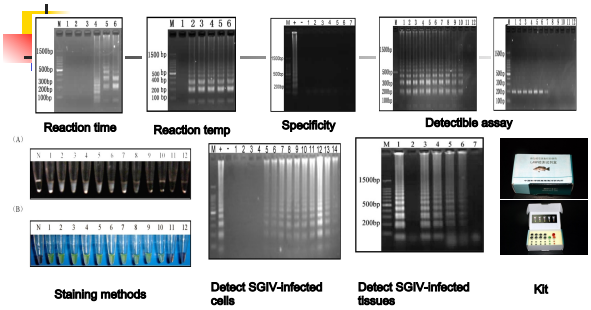
LAMP assay



Mao XL, et al., 2008. J App Microbial.

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3. Inactivated vaccine against SGIV

Inactivated whole virus：BPL treated virus for 24h at 4° C

BPL 浓度	处理 时间	盲传三代结果 1	2	3
1：250	6h	+	+	+
	12h	-	-	-
	24h	-	-	-
1：500	6h	+	+	+
	12h	-	-	-
	24h	-	-	-
1：1000	48h	+	+	+
	60h	-	+	+
	72h	-	+	+
1:2000	48h	+	+	+
	60h	+	+	+
	72h	+	+	+

（+ positive CPE，-negative CPE）

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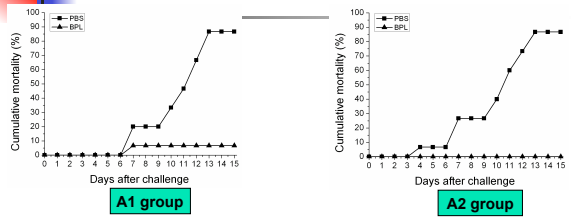
The vaccine showed good potential to protect grouper from SGIV infection



Treat	Survival	Relative prot
A1-PBS	13.33%	-
A1-BPL	93.33%	92.31

Treat	Survival	Relative prot
A2-PBS	13.33%	-
A2-BPL	100%	100

每尾石斑鱼腹腔注射0.1mL (10^6 TCID50)灭活疫苗，每组免疫30尾，对照组注射同样剂量的PBS，各分2个缸。15天后攻毒，腹腔注射接种 SGIV，统计15天

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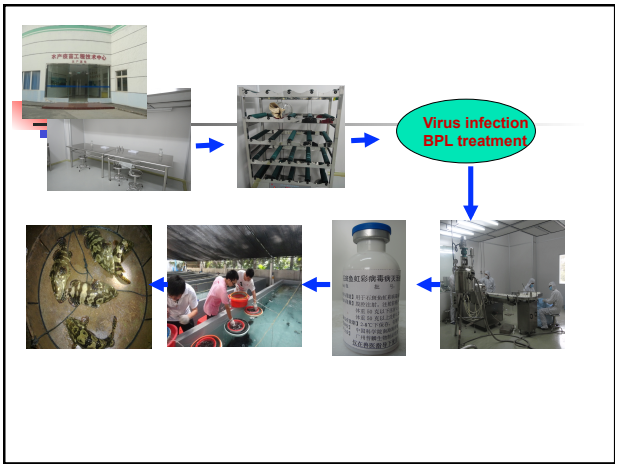
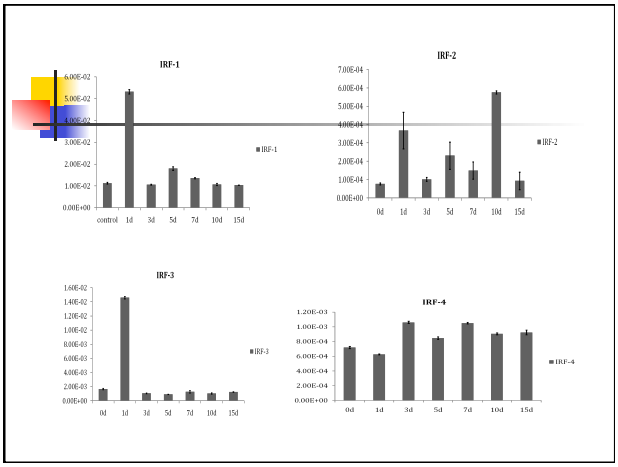
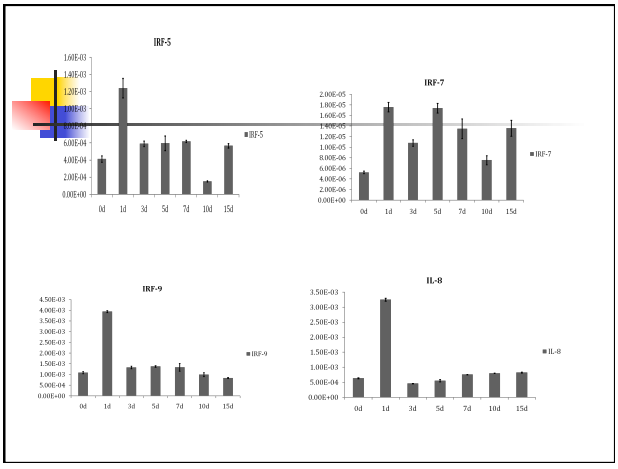
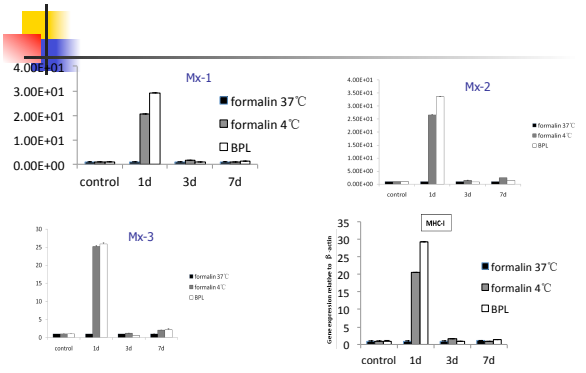

Virus infection
BPL treatment


IRF-1
IRF-2
IRF-3
IRF-4


IRF-5
IRF-7
IRF-9
IL-8



Perspective Work

- ❖ Interaction & regulations between virus and host
- ❖ Development of vaccines and antiviral strategies
- ❖ Novel diagnostics

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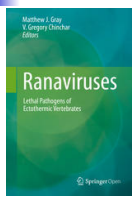
Reference book



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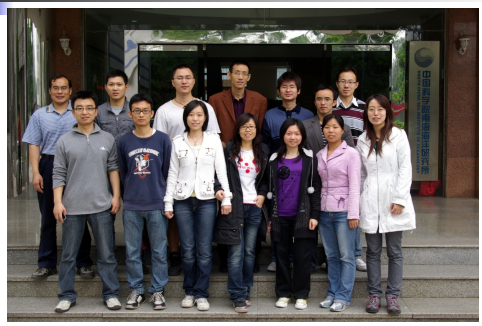

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Thank you

谢 谢