Journal of Microbiology, Immunology and Infection (2017) 50, 747–754

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Review Article

The *p53* gene with emphasis on its paralogues in mosquitoes

Tien-Huang Chen [a], Yi-Jun Wu [b], Jiun-Nan Hou [b], Cheng-Hsun Chiu [c,d], Wei-June Chen [a,b,c,*]

[a] *Departments of Public Health and Parasitology, Chang Gung University, Kwei-San, Tao-Yuan 33332, Taiwan*
[b] *Graduate Institute of Biomedical Sciences, Chang Gung University, Kwei-San, Tao-Yuan 33332, Taiwan*
[c] *Molecular Infectious Disease Research Center, Chang Gung Memorial Hospital, Kwei-San, Tao-Yuan, Taiwan*
[d] *Division of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Children's Hospital, Chang Gung University College of Medicine, Kwei-San, Tao-Yuan, Taiwan*

Received 27 February 2017; received in revised form 31 May 2017; accepted 21 June 2017
Available online 29 June 2017

KEYWORDS
p53 homologue;
Paralogue;
Mosquitoes;
Phylogeny;
Cell survival

Abstract The *p53* gene is highly important in human cancers, as it serves as a tumor-suppressor gene. Subsequently, two p53 homologues, *i.e.*, p73 and p63, with high identity of amino acids were identified, leading to construction of the p53 family. The *p53* gene is highly important in human cancer because it usually transcribes genes that function by causing apoptosis in mammalian cells. In contrast, p63 and p73 tend to be more important in modulating development than inducing cell death, even though they share similar protein structures. Relatively recently, p53 was also identified in mosquitoes and many other insect species. Uniquely, its structure lacks the sterile alpha motif domain which is a putative protein-protein interaction domain and exclusively exists at the C-terminal region in p73 and p63 in mammals. A phylogenetic analysis revealed that the *p53* gene derived from mosquitoes is composed of two paralogues, p53-1 and p53-2. Of these, only p53-2 is responsively up-regulated by dengue 2 virus (DENV2) in C6/36 cells which usually survive the infection. This indicates that the *p53* gene is closely related to DENV infection in mosquito cells. The specific significance of p53-2's involvement in cell survival from virus-induced stress is described and briefly discussed in this report.

* Corresponding author. Departments of Public Health and Parasitology, Chang Gung University, Kwei-San, Tao-Yuan 33332, Taiwan. Fax: +886 3 2118408.
E-mail address: wjchen@mail.cgu.edu.tw (W.-J. Chen).

http://dx.doi.org/10.1016/j.jmii.2017.06.006

Background

The eukaryotic cell cycle is normally divided into four distinct phases, G_1, S, G_2, and M; its progression can be downregulated through a p53-depedent pathway when stress-induced DNA damage occurs.[1] The *p53* gene in mammal cells was demonstrated to be a critical mediator of the apoptotic response to DNA double-strand (ds) breaks via the transcriptional activation of proapoptotic genes.[2] Therefore, the genomic integrity of a cell population or organism can be maintained. Mutation of the *p53* gene and/or a functional defect in the p53 pathway usually results in ineffectiveness in causing apoptosis which was found in most human tumor cells.[3] About 50% of cancer cases are estimated to possess a mutation of the *p53* gene, and almost all cancers exhibit inactivity of p53.[4] p53 is genetically conserved in a broad spectrum from mammals to lower invertebrates.[5] Two more homologs, *i.e.*, p63 and p73, were subsequently discovered as additional members of the p53 family.[6] The common ancestor gene of p53 family members is supposed to be the first gene that duplicated to produce a p53 gene and a p63/p73 ancestor in cartilaginous fish.[7] Bony fish and higher vertebrates contain all three genes with diverse functions despite their possessing preserved structural features.[8]

The p53 family

The basic structure of p53 is composed of four conserved domains (Fig. 1), including an amino-terminal transactivation domain (TAD) consisting of a proline-rich domain (PR), a central DNA-binding domain (DBD), and a carboxy-terminal oligomerization domain (OD).[9] The TAD is highly associated with the cell fate, presumably governing genes involved in cellular senescence, DNA editing, and repair pathways.[10] The DBD is located in the central region and is the target of most p53 mutations found in human cancers.[9] The OD contains a nuclear export signal (NES) and contributes to form a dimer of two dimers of p53 in structure.[9] The sterile alpha motif (SAM) is a putative protein-protein interaction domain that exclusively exists in the C-terminal region of p63 and p73.[11] The SAM domain is necessary to stabilize the OD structure in both p63 and p73.[11] In many proteins, the SAM domain is involved in signaling and transcription, providing a structure which appropriately binds phosphotyrosine phosphatase and initiates downstream signaling events.[12]

It was reported that p53 independently duplicates, and therefore, it is a divergent ancestral gene from p63 and p73, although they have shared structural identities to each other.[13] Comparing gene compositions, p63 and p73 are more similar to each other than each of them is to p53.[7] As a result, p63 and p73 are thought to have more-ancient roots and are likely to be the ancestors of p53.[6] However, there is increasing evidence showing that they have shared, overlapping functions. For instance, they may commonly induce cell-cycle arrest and apoptosis in cells.[14] Nevertheless, distinct functions among them are also reported, such as involvement in regulating stress responses to suppress tumors, ectoderm development, and both.[15] DNA damage usually activates p53 but not p63 or p73[14], further revealing the existence of different physiological functions among members of the p53 family. In a study using p63 and p73 knockout in mice, developmental abnormalities but not cancer susceptibility were observed.[16] Another study also showed that the combined loss of p63 and p73 caused failure of apoptosis in cells containing functional p53 in response to DNA damage.[17] Mutations of p63 and p73 rarely being found in human cancers reflects that p63 and p73 are more important in modulating development than in inducing cancer.[4] Nevertheless, p53, p73, and p63 may interact with each other, as p53 mutants with loss of the tumor-suppressing capacity were reported to inactivate p73.[15,18] Studies on their interactions are required for further clarification of relationships among them.[10]

In a cell in a resting status, p53 is localized in the cytoplasm, while it accumulates in the nucleus following stress and functions as a transcription factor.[19] According to a genome-wide investigation, 149 putative new p53 target genes were highly associated with cancer.[20] Another study further revealed that at least 125 protein-coding genes and noncoding RNAs are transcriptional targets directly regulated by p53 under a wide range of stress signals in cells.[21] These results suggest that p53 is responsive to various stresses induced by chemical mutagens, irradiation, viral infections, etc.[1] In addition, diverse transcriptional co-regulators may be recruited to regulate cellular RNA

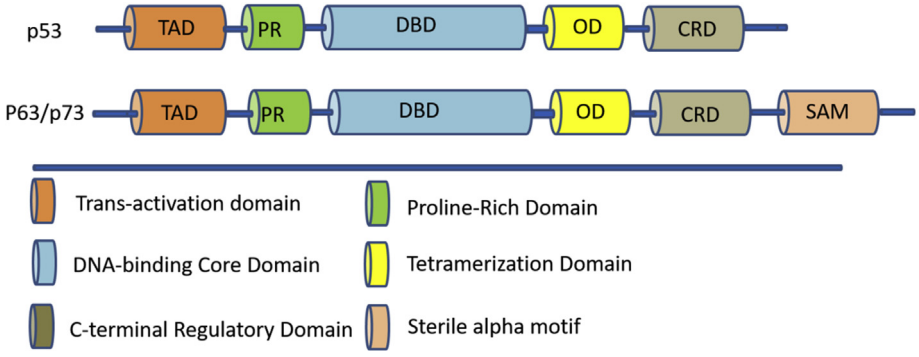

Fig. 1. Schematic structures of p53 and p63/p73 members of the p53 family. All of them are composed of a transactivation domain (TAD), proline-rich domain (PR), DNA-binding core domain (DBD), oligomerization domain (OD), and C-terminal regulatory domain (CRD) while an additional sterile alpha motif (SAM) exclusively exists at the C-terminus of p63 and p73.

polymerase II activity by stress-activated p53 modulation of transcription initiation and elongation at target gene promoters.[22] The promoter selectivity and transcriptional regulation of p53 target genes are assumed to be the effects of diverse stresses.[23] It was noted that low levels of stress-induced DNA damage can be repaired unless the damage persists and becomes extensive.[24]

p53 in insects

Genes in the p53 family are not detectable in prokaryotes or yeast.[8] However, the exist in single-celled choanoflagellates which are believed to be the most primitive invertebrates possessing genes belonging to the p53 family.[25] *Caenorhabditis elegans* was reported to have a homologue of mammalian p53, indicating that this primitive multiple-celled organism also requires p53 for DNA damage-induced apoptosis in its germline.[26] However, it does not affect programmed cell death during development of the worm,[27] suggesting that p53-mediated transcriptional regulation in *C. elegans* follows an ancestral function as is now known invertebrates. Nowadays, p53 is known to be prevalent in a wide range of invertebrates including insects.[27] p53 from invertebrates/insects is structurally similar to that in vertebrates and mammals, consisting of a TAD (including the neighboring proline-rich region), DBD, and OD in sequence.[28] Very importantly, p53 found in invertebrates lacks the SAM domain, although the full length of the gene sequence generally shares similarity with p63/p73 homologues.[11] In addition, p53 of insects/invertebrates usually has a molecular weight lower than that mammalian p53; for instance, it is 41.2 kDa in *Bombyx mori* and 42.5 kDa in *Spodoptera frugiperda*.[29]

Thus far, p63/p73 homologues have not been identified in *Drosophila* or other insects although the biological functions of *Drosophila* p53 family genes have been mentioned to be closer to the vertebrate p63-like family genes. As a result, the *Drosophila p53* gene is believed to have originated from a p53-like ancestor but not a duplicate of p67/p73 which is supposed to be the common ancestor of *p53* genes in vertebrates.[37] However, p53 identified from squid was observed to be more similar to human p67/p73 in gene sequences.[30] Meanwhile, p53 found in mollusks has a structure either with or without the SAM domain.[31] This implies that p53 of invertebrates or insects may be linked to p67/p73.

p53 paralogues in mosquitoes

The *p53* gene has been identified in a number of insects, including species in the orders of Homoptera, Hymenoptera, Coleoptera, Lepidoptera, and Diptera.[29] However, information with respect to mosquito p53 is limited, while it has been widely investigated in the Diptera, particularly *Drosophila*.[27] Mosquitoes are important members of the Diptera; which serve as vectors of a great number of human diseases, such as malaria, filariasis, and various viral infections. Thus far, p53 has been identified in more than 20 species of mosquitoes including 19 *Anopheles* species in the subfamily Anophelinae and three species (*Aedes aegserve*, *Ae. albopictus*, and *Culex pipiens quinquefasciatus*) in the subfamily Culicinae (available at the VectorBase webpage: 8～https://www.vectorbase.org/Culex_quinquefasciatus/Gene/Compara_Ortholog?db=core;g=CPIJ002758;r=supercont3.36:1214079-1215512;t=CPIJ002758-RA). Of these, *Ae. aegypti* and probably *Ae. albopictus* are considered to be principal vectors of dengue fever (DENV), Zika fever, and Chikungunya that are prevalent in most tropical countries while *Cx. quinquefasciatus* is the main vector of bancroftian filariasis.[32,33] Therefore, relationships of arboviruses or other arthropod-borne pathogens with their mosquito hosts have attracted attention, as the fate of mosquito cells with respect to infections may determine the role of mosquitoes as transmission vectors. According to a previous report, p53 is one of the mosquito genes which positively responds to DENV infection.[34]

A phylogenetic tree constructed on the basis of p53 amino acid sequences derived from 22 species of mosquitoes, a panel of insects, and the snail *Biomphalaria glabrata* (the outgroup) is shown at the VectorBase webpage: https://www.vectorbase.org/Multi/GeneTree/Image?gt=VBGT00190000010787. According to the constructed tree, p53 of ticks is evolutionarily distant from those of insects. *p53* genes identified in non-dipteran insects, including bed bugs (*Cimex lectularius*) and cone-nose bugs (*Rhodnius prolixus*) (Hemiptera), and body lice (*Pediculus humanis*) (Anoplura), are grouped in a single clade with a distant evolutionary relationship from the dipterans. Uniquely, unlike those from vertebrates, *p53* genes derived from currently selected insects are divided into two distinct clades. The first clade (at the upper portion in the tree) contains one p53 homologue derived from all mosquitoes and the sand fly *Lutzomyia longipalpis*, both of which belong to the suborder Nematocera in the order Diptera. Species belonging to the suborder Brachycera (*Musca domestica*, *Stomoxys calcitrans*, *Drosophila melanogaster*, and *Glossina* spp.) in the order Diptera are also in this clade. The second clade (at the lower portion of the tree) contains the other p53 homologues also derived from mosquitoes and a few genetically distant species of insects, including several species of tsetse flies (*Glossina* spp.), *C. lectularius*, *R. prolixus*, and *P. humanis*. Among them, *Glossina morsitans* is the only species of currently known insects that possesses two clades of p53 other than mosquitoes.

Isoforms of each member of the p53 family have been identified from many organisms[35]; these may be created through multiple splicing events, alternative promoters, and different alternative initiation events of translation.[18] Due to divergent biological properties among isoforms of p53, a complex network of functions may thus be constructed.[36] Isoforms may further be referred to as paralogues because of a predominance of substitutions.[37] Hypothetically, they are descendants of two different copies of the same gene through a duplication event in the common ancestor genome.[38] As mentioned, all *p53* genes identified from mosquitoes and *G. morsitans* include two clades which are now referred to as two paralogues, *i.e.*, p53-1 and p53-2. Interestingly, most insects other than mosquitoes contain only a single homologue. With a few exceptions, p53 paralogues exclusively exist in the suborder Nematocera but not the suborder Brachycera, both of which taxonomically belong to the order Diptera.

Molecularly, p53-1 and p53-2 isoforms can be simultaneously identified in *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and *Anopheles* species of mosquitoes. Based on a simplified phylogenetic tree constructed by the Neighbor-joining method (Fig. 2a), both p53-1 and p53-2 are genetically closer between *Aedes* and *Culex*; however, they are more distant to *Anopheles* homologues. *p53* genes from *D. melanogaster*, *Ceratitis capitate*, and

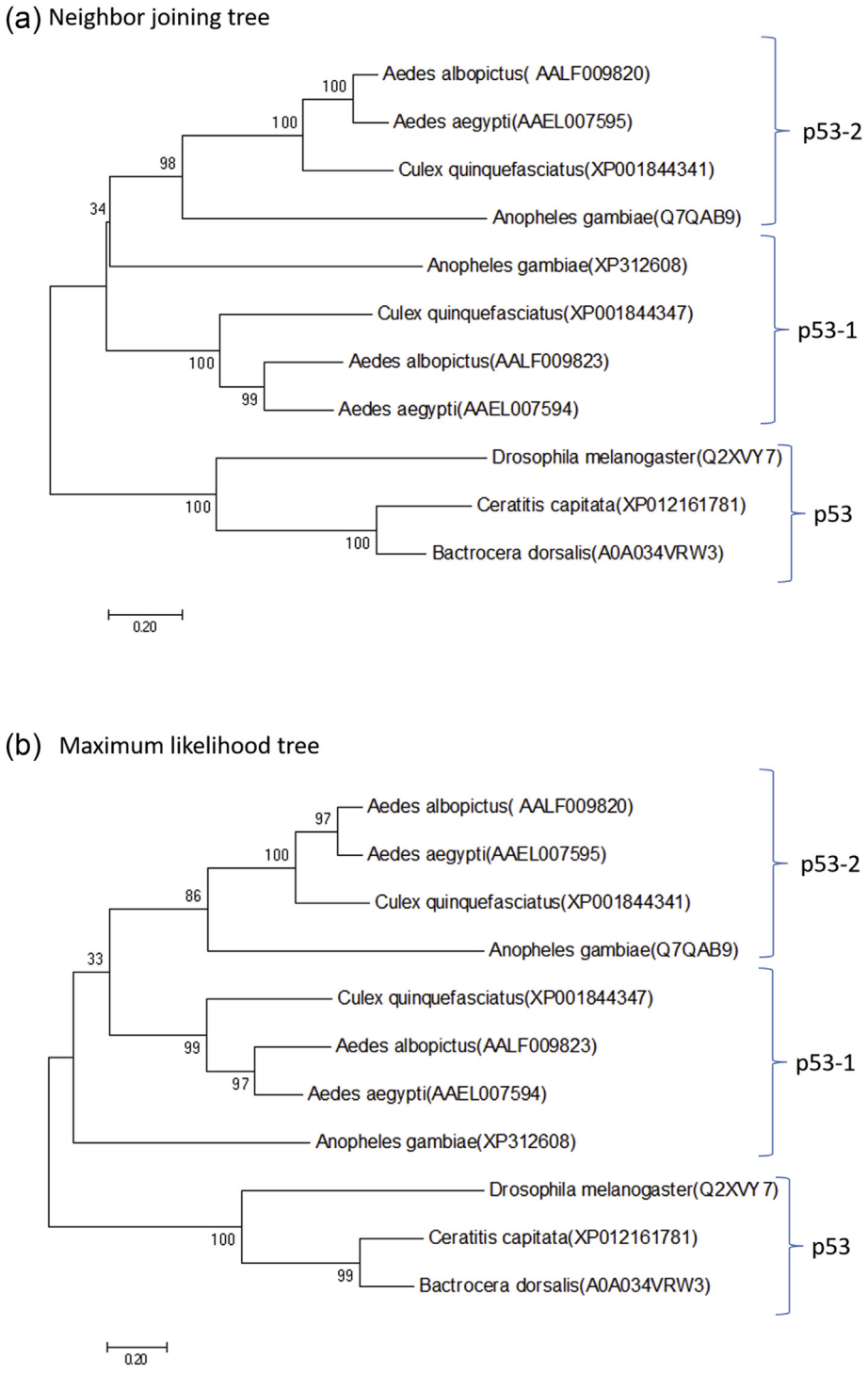

Fig. 2. Deduced amino acid sequence-based phylogenetic trees for selected p53 homologues were constructed by Neighbor-joining (a) and maximum-likelihood (b) methods, based on p53 homologues derived from seven dipterans including four species of mosquitoes (suborder Nematocera) and three species of flies (suborder Brachycera). All four mosquitoes possessed two paralogues of p53 (p53-1 and p53-2), while there was a single homologue in flies. Numbers on the branches are bootstrap proportions (1000 replicates).

Table 1 Distance estimations[a] between aligned p53 paralogues (p53-1 and p53-2) derived from the deduced amino acid sequences of three species of culicine mosquitoes (*Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus*) and one anopheline species (*Anopheles gambiae*).

		p53-1				p53-2			
		Aedes aegypti (AAEL007594)	Aedes albopictus (AALF010441)	Culex quinquefasciatus (CPIJ002764)	Anopheles gambiae (AGAP002352)	Aedes aegypti (AAEL007595)	Aedes albopictus (AALF009820)	Culex quinquefasciatus (CPIJ002758)	Anopheles gambiae (AGAP004319)
p53-1	Aedes aegypti (AAEL007594)	—							
	Aedes albopictus (AALF010441)	0.677	—						
	Culex quinquefasciatus (CPIJ002764)	0.501	0.524	—					
	Anopheles gambiae (AGAP002352)	0.216	0.223	0.223	—				
p53-2	Aedes aegypti (AAEL007595)	0.280	0.252	0.263	0.185	—			
	Aedes albopictus (AALF009820)	0.296	0.268	0.263	0.189	0.823	—		
	Culex quinquefasciatus (CPIJ002758)	0.276	0.263	0.262	0.186	0.63	0.617	—	
	Anopheles gambiae (AGAP004319)	0.198	0.235	0.22	0.188	0.243	0.241	0.252	—

[a] The sequences used for the analysis were not automatically aligned before the procedure we ran. BioEdit offers ClustalW as a means of computer-aided alignment. The numbers of amino acids analyzed were 385–459, depending on the species in question.

Bactrocera dorsalis (all belonging to the suborder Brachycera) were used as outgroups and were shown to be the most distant from those of mosquitoes, either p53-1 or p53-2.[39] The phylogenetic tree constructed by the maximum-likelihood method reveals the same trend (Fig. 2b). It reflects that p53 has evolved along with the speciation of mosquitoes, from which at least two paralogues were created. Their individual functions remain as an attractive topic for study in the future.

The *p53* gene of mosquitoes is composed of 431–459 amino acids according to VectorBase. An identity matrix showing the proportions of identical residues within or between p53 paralogues from three species of culicine and one species of anopheline mosquitoes was produced (Table 1). The estimation of their evolutionary distances revealed that the p53-1 paralogue of *Ae. aegypti* (AAEL007594) shared 67.7% similarity with that from *Ae. albopictus* (AALF009823) and 50.1% with that from *Cx. quinquefasciatus* (CPIJ002764). However, it was as low as 21.6% in similarity with that from *Anopheles gambiae* (AGAP002352), the representative species of anopheline mosquitoes. The p53-2 paralogue from *Ae. aegypti* (AAEL007595) shared 82.3% similarly with that from *Ae. albopictus* (AALF009820) and 63.0% with that from *Cx. quinquefasciatus* (CPIJ002758). However, it had only 24.3% similarity compared to that from *An. gambiae* (AGAP004319). Looking at the genetic distance between the p53-1 and p53-2 paralogues, there was a 28.0% similarity of *Ae. aegypti*, 26.8% with *Ae. albopictus*, 26.2% with *Cx. quinquefasciatus*, and 18.8% with *An. gambiae*. This suggests that evolutionary distances between the p53-1 and p53-2 paralogues are relatively great regardless of which species of mosquito were their origins. Looking at the same comparison focusing on the predicted DBD, a similar evolutionary trend was shown at the base of the p53-1 and p53-2 paralogues derived from the four mosquito species (Table 2). Results revealed that there was only 51.3% similarity between the predicated DBDs of the p53-1 and p53-2 paralogues even though they were all derived from *Ae. aegypti*.

Potential functions of the p53 paralogues in mosquitoes

It is beyond doubt that arboviruses modulate gene expressions in mosquitoes in response to stresses induced by insecticides.[40] Alterations in gene expressions are also shown in the midgut of female *Cx. quinquefasciatus* exposed to blood meals containing West Nile virus (WNV).[41] This indicates that arboviruses may activate mosquito genes which interact with invading viruses and/or biological functions of mosquitoes themselves.[42] Apoptosis-related genes are usually not upregulated in susceptible strains of *Aedes* mosquitoes with DENV infection.[43] This implies that arboviral infection does not cause a high level of endoplasmic reticular (ER) stress that is able to induce apoptosis of mosquito cells with infections. According to a meta-analysis of studies across a range of mosquito/virus systems, on some occasions, arboviruses may also be deleterious to their mosquito vectors,[44] leading to a function of determining vector competence.[45] More often, arboviruses rarely cause apoptosis even though they are pathogens harmful to humans due to their co-evolution towards a

Table 2 Distance estimations[a] of the predicted DNA-binding domain between aligned p53 paralogues (p53-1 and p53-2) derived from the deduced amino acid sequences of three species of culicine mosquitoes (*Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus*) and one anopheline species (*Anopheles gambiae*).

		p53-1				p53-2			
		Aedes aegypti (AAEL007594)	Aedes albopictus (AALF010441)	Culex quinquefasciatus (CPIJ002764)	Anopheles gambiae (AGAP002352)	Aedes aegypti (AAEL007595)	Aedes albopictus (AALF009820)	Culex quinquefasciatus (CPIJ002758)	Anopheles gambiae (AGAP004319)
p53-1	Aedes aegypti (AAEL007594)	—							
	Aedes albopictus (AALF010441)	0.859	—						
	Culex quinquefasciatus (CPIJ002764)	0.773	0.708	—					
	Anopheles gambiae (AGAP002352)	0.387	0.392	0.407	—				
p53-2	Aedes aegypti (AAEL007595)	0.513	0.491	0.484	0.357	—			
	Aedes albopictus (AALF009820)	0.535	0.508	0.484	0.357	0.921	—		
	Culex quinquefasciatus (CPIJ002758)	0.508	0.481	0.468	0.357	0.804	0.798	—	
	Anopheles gambiae (AGAP004319)	0.333	0.343	0.326	0.312	0.387	0.398	0.382	—

[a] The sequences used for the analysis were not automatically aligned before the procedure we ran. BioEdit offers ClustalW as a means of computer-aided alignment. The numbers of amino acids analyzed were 385–459, depending on the species in question.

benign relationship between the virus and its host vectors.[46] In such cases, mosquito cells may operate antiviral mechanisms or host defense, leading to a great help for self-protection of infected cells.[42,47]

Both antioxidant defense and antiapoptotic effects were reported to be induced in mosquito cells with DENV infection via reducing the accumulation of intracellular reactive oxygen species (ROS).[34,42] ROS are now known to act as upstream signals triggering p53 and its downstream factors in association with apoptosis.[48] p53 target genes, such as reper in *Drosophila* or Michelob_x in mosquito cells, may be transactivated following infection with pathogenic viruses and certain arboviruses,[49] leading to a cell-killing effect.[50] Despite apoptosis also appearing in mosquito cells with arboviruses,[46] the p53-mediated signaling pathway may play a significant role in protecting infected cells. Recently, we demonstrated that p53-2 (with a 2.27-fold increase in infected cells), but not p53-1 (with a 1.24-fold increase), was significantly upregulated in response to DENV infection for 24 h in C6/36 cells ($p < 0.05$; Student's *t*-test) (Fig. 3). This reflects that p53-2 in *Ae. albopictus* cells plays a more important role of overcoming oxidative stress and surviving DENV infection than does p53-1. When p53-2 was knocked down in C6/36 cells, the apoptosis rate was obviously enhanced at 24 h post-infection by the DENV (W. J. Chen, unpublished data). Presumably, p53-2 functions to create an environment beneficial for viral replication in mosquito cells. In the meantime, it helps mosquito cells survive DENV infection. Despite more results are needed, we have preliminarily confirmed a critical role of p53-2 in reducing ROS accumulation and the death rate of mosquito cells with DENV infection. Undoubtedly, a higher survival rate of mosquito cells is a prerequisite for prosperous production of viral progeny. This feature may account for the benign outcome of mosquito cells with infections of most arboviruses which are thus transmitted efficiently in nature.

Conclusions

Genes in the p53 family, *i.e.*, p53, p63, and p73, have been identified in most eukaryotic vertebrates. Of these, p53 is

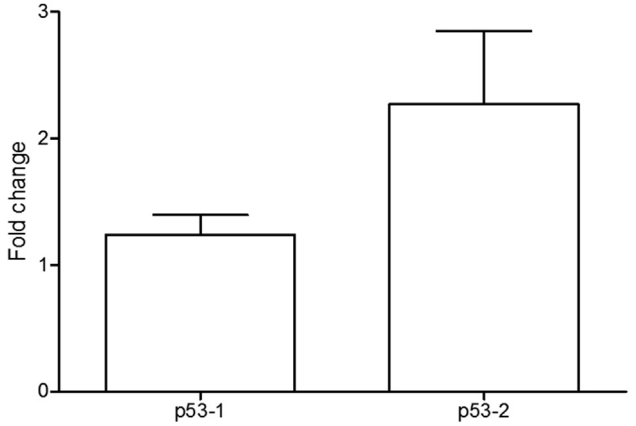

Fig. 3. p53-1 and p53-2 expression levels in *Aedes albopictus*-derived C6/36 cells in response to dengue 2 virus (DENV2) infection for 24 h. Results show that only p53-2, but not p53-1, was upregulated by DENV2 in mosquito cells.

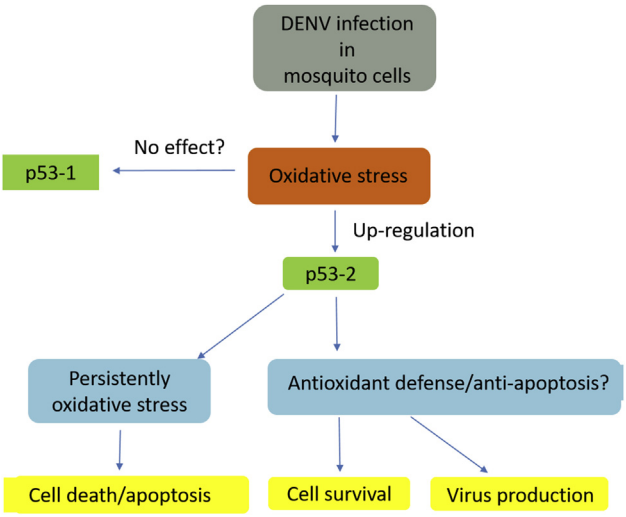

Fig. 4. The p53 pathway in mosquito cells with dengue 2 virus (DENV2) infection. DENV2-induced oxidative stress may result in upregulation of p53-2 in mosquito cells. Infected cells may end up dying if oxidative stress persists. On the other hand, upregulation of p53-2 may be involved in antioxidant defense and antiapoptotic effects, leading to the survival of infected cells in which the virus continually replicates.

believed to regulate apoptosis, while p63 and p73 tend to be involved in development. Thus far, p53 is the only member found in invertebrates; however, it lacks the SAM domain at the 3′-terminus. p53 identified from mosquitoes was demonstrated to consist of two paralogues (p53-1 and p53-2). However, only p53-2 is upregulated in response to ER stress induced by DENV infection and is very likely involved in protecting mosquito cells. Its target genes are primarily those responsible for antioxidant defense and antiapoptotic effects which were found to be induced (Fig. 4). In conclusion, p53-2 plays a role in mediating a balance between viral replication and mosquito survival, facilitating an elucidation of how arboviruses can be amplified in and successfully transmitted by mosquito vectors.

Conflicts of interest

All contributing authors declare no financial interests related to the material in the manuscript.

Acknowledgments

This work was supported by a grant from Chang Gung Memorial Hospital (CMRPD1E0201～2 and CMRPD1F0321) and partly from the Ministry of Science and Technology, Executive Yuan, Taiwan (NSC103-2320-B-182-029-MY3).

References

1. North S, Hainaut P. p53 and cell-cycle control: a finger in every pie. *Pathol Biol* 2000;**48**(3):255—70.
2. Chakraborty R, Li Y, Zhou L, Golic KG. Corp regulates P53 in Drosophila melanogaster via a negative feedback loop. *PLoS Genet* 2015;**11**(7). e1005400.
3. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science* 1991;**253**(5015):49—53.
4. DeLaurenzi V, Melino G. Evolution of functions within the p53/p63/p73 family. *Ann N Y Acad Sci* 2000;**926**:90—100.
5. Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 database: new online mutation analysis and recommendations to users. *Hum Mutat* 2002;**19**(6):607—14.
6. Morgunkova AA. The p53 gene family: control of cell proliferation and developmental programs. *Biochem* 2005;**70**(9): 955—71.
7. Strano S, Rossi M, Fontemaggi G, Munarriz E, Soddu S, Sacchi A, et al. From p63 to p53 across p73. *FEBS Lett* 2001;**490**(3): 163—70.
8. Belyi VA, Ak P, Markert E, Wang H, Hu W, Puzio-Kuter A, et al. The origins and evolution of the p53 family of genes. *Cold Spring Harb Perspect Biol* 2010;**2**(6). a001198.
9. Wang P, Reed M, Wang Y, Mayr G, Stenger JE, Anderson ME, et al. p53 domains: structure, oligomerization, and transformation. *Mol Cell Biol* 1994;**14**(8):5182—91.
10. Walker CW, VanBeneden RJ, Muttray AF, Bottger SA, Kelley ML, Tucker AE, et al. p53 superfamily proteins in marine bivalve cancer and stress biology. *Adv Mar Biol* 2011;**59**:1—36.
11. Thanos CD, Bowie JU. p53 Family members p63 and p73 are SAM domain-containing proteins. *Protein Sci* 1999;**8**(8): 1708—10.
12. Levrero M, DeLaurenzi V, Costanzo A, Gong J, Melino G, Wang JY. Structure, function and regulation of p63 and p73. *Cell Death Differ* 1999;**6**(12):1146—53.
13. Zhang J. Evolution by gene duplication: an update. *Trends Ecol Evol* 2003;**18**(6):292—8.
14. Lohrum MA, Vousden KH. Regulation and function of the p53-related proteins: same family, different rules. *Trends Cell Biol* 2000;**10**(5):197—202.
15. Levrero M, DeLaurenzi V, Costanzo A, Gong J, Wang JY, Melino G. The p53/p63/p73 family of transcription factors: overlapping and distinct functions. *J Cell Sci* 2000;**113**(10): 1661—70.
16. Moll UM, Slade N. p63 and p73: roles in development and tumor formation. *Mol Cancer Res* 2004;**2**(7):371—86.
17. Flores ER, Tsai KY, Crowley D, Sengupta S, Yang A, McKeon F, et al. p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. *Nature* 2002;**416**(6880):560—4.
18. Murray-Zmijewski F, Lane DP, Bourdon JC. p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. *Cell Death Differ* 2006;**13**(6): 962—72.
19. Marine JC. p53 stabilization: the importance of nuclear import. *Cell Death Differ* 2010;**17**(2):191—2.
20. Menendez D, Nguyen TA, Freudenberg JM, Mathew VJ, Anderson CW, Jothi R, et al. Diverse stresses dramatically alter genome-wide p53 binding and transactivation landscape in human cancer cells. *Nucleic Acids Res* 2013;**41**(15): 7286—301.
21. Riley T, Sontag E, Chen P, Levine A. Transcriptional control of human p53-regulated genes. *Nat Rev Mol Cell Biol* 2008;**9**(5): 402—12.
22. Laptenko O, Prives C. Transcriptional regulation by p53: one protein, many possibilities. *Cell Death Differ* 2006;**13**(6):951—61.
23. Beckerman R, Prives C. Transcriptional regulation by p53. *Cold Spring Harb Perspect Biol* 2010;**2**(8). a000935.
24. Bensaad K, Vousden KH. Savior and slayer: the two faces of p53. *Nat Med* 2005;**11**(12):1278—9.
25. Rutkowski R, Hofmann K, Gartner A. Phylogeny and function of the invertebrate p53 superfamily. *Cold Spring Harb Perspect Biol* 2010;**2**(7). a001131.

26. Schumacher B, Hofmann K, Boulton S, Gartner A. The C. elegans homolog of the p53 tumor suppressor is required for DNA damage-induced apoptosis. *Curr Biol* 2001;**11**(21):1722—7.
27. Jin S, Martinek S, Joo WS, Wortman JR, Mirkovic N, Sali A, et al. Identification and characterization of a p53 homologue in Drosophila melanogaster. *Proc Natl Acad Sci U S A* 2000;**97**(13):7301—6.
28. Ollmann M, Young LM, DiComo CJ, Karim F, Belvin M, Robertson S, et al. Drosophila p53 is a structural and functional homolog of the tumor suppressor p53. *Cell* 2000;**101**(1):91—101.
29. Huang N, Clem RJ, Rohrmann GF. Characterization of cDNAs encoding p53 of Bombyx mori and Spodoptera frugiperda. *Insect Biochem Mol Biol* 2011;**41**(8):613—9.
30. Nordstrom W, Abrams JM. Guardian ancestry: fly p53 and damage-inducible apoptosis. *Cell Death Differ* 2000;**7**(11):1035—8.
31. VanBeneden RJ, Walker CW, Laughner ES. Characterization of gene expression of a p53 homologue in the soft-shell clam (Mya arenaria). *Mol Mar Biol Biotechnol* 1997;**6**(2):116—22.
32. Likos A, Griffin I, Bingham AM, Stanek D, Fische M, White S, et al. Local mosquito-borne transmission of Zika virus - miami-dade and broward counties, Florida, June-August 2016. *MMWR Morb Mortal Wkly Rep* 2016;**65**(38):1032—8.
33. Tolle MA. Mosquito-borne diseases. *Curr Probl Pediatr Adolesc Health Care* 2009;**39**(4):97—140.
34. Chen TH, Tang P, Yang CF, Kao LH, Lo YP, Chuang CK, et al. Antioxidant defense is one of the mechanisms by which mosquito cells survive dengue 2 viral infection. *Virology* 2011;**410**(2):410—7.
35. Freed-Pastor WA, Prives C. Mutant p53: one name, many proteins. *Genes Dev* 2012;**26**(12):1268—86.
36. Dotsch V, Bernassola F, Coutandin D, Candi E, Melino G. p63 and p73, the ancestors of p53. *Cold Spring Harb Perspect Biol* 2010;**2**(9). a004887.
37. Spitzer M, Lorkowski S, Cullen P, Sczyrba A, Fuellen G. IsoSVM—distinguishing isoforms and paralogs on the protein level. *BMC Bioinforma* 2006;**7**:110.
38. Brinkman FS, Leipe DD. Phylogenetic analysis. *Methods Biochem Anal* 2001;**43**:323—58.
39. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;**33**(7):1870—4.
40. Games PD, Alves SN, Katz BB, Tomich JM, Serrao JE. Differential protein expression in the midgut of Culex quinquefasciatus mosquitoes induced by the insecticide temephos. *Med Vet Entomol* 2016;**30**(3):253—63.
41. Smartt CT, Richards SL, Anderson SL, Erickson JS. West Nile virus infection alters midgut gene expression in culex pipiens quinquefasciatus say (Diptera: Culicidae). *Am J Trop Med Hyg* 2009;**81**(2):258—63.
42. Chen TH, Lo YP, Yang CF, Chen WJ. Additive protection by antioxidant and apoptosis-inhibiting effects on mosquito cells with dengue 2 virus infection. *PLoS Negl Trop Dis* 2012;**6**(4):e1613.
43. Ballarin L, Cammarata M. *Lessons in immunity: from single-cell organisms to mammals*. Academic Press; 2016.
44. Lambrechts L, Scott TW. Mode of transmission and the evolution of arbovirus virulence in mosquito vectors. *Proc Biol Sci* 2009;**276**(1660):1369—78.
45. Clem RJ. Arboviruses and apoptosis: the role of cell death in determining vector competence. *J Gen Virol* 2016;**97**(5):1033—6.
46. O'Neill K, Olson BJ, Huang N, Unis D, Clem RJ. Rapid selection against arbovirus-induced apoptosis during infection of a mosquito vector. *Proc Natl Acad Sci U S A* 2015;**112**(10):E1152—61.
47. Lopez-Montero N, Risco C. Self-protection and survival of arbovirus-infected mosquito cells. *Cell Microbiol* 2011;**13**(2):300—15.
48. Liu B, Chen Y, St Clair DK. ROS and p53: a versatile partnership. *Free Radic Biol Med* 2008;**44**(8):1529—35.
49. Liu B, Becnel JJ, Zhang Y, Zhou L. Induction of reaper ortholog mx in mosquito midgut cells following baculovirus infection. *Cell Death Differ* 2011;**18**(8):1337—45.
50. White K, Tahaoglu E, Steller H. Cell killing by the Drosophila gene reaper. *Science* 1996;**271**(5250):805—7.