Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 5 Issue 12 December 2022

Research Article

In silico Analysis of the N-terminal Region of Lepidopteran Cadherins

Mendoza-Osorno A[1,2], Olguín-Ruiz GE[1], Sánchez-Vallejo CJ[1], Pérez-Díaz JM[3] and Guerrero GG[4]*

[1]*Instituto Politécnico Nacional, Departamento de Bioquímica, Laboratorio de Genetica Molecular, CDMX, Mexico*

[2]*Universidad Nacional Autónoma de México, Facultad de Química, Programa de Licenciatura en Química, Circuito de Ciudad Universitaria, CDMX, Mexico*

[3]*Universidad Autónoma de Zacatecas, Campus II, Unidad Académica de Matemáticas, Zacatecas, Zacatecas, Mexico*

[4]*Universidad Autónoma de Zacatecas, Campus II, Unidad Académica de Ciencias Biológicas, Laboratorio de Inmunobiologia, Zacatecas, Zac, MX, Mexico*

***Corresponding Author:** Guerrero GG, Universidad Autónoma de Zacatecas, Campus II, Unidad Académica de Ciencias Biológicas, Laboratorio de Inmunobiologia, Zacatecas, Zac, MX, Mexico.

DOI: 10.31080/ASMI.2022.05.1176

Received: November 02, 2022

Published: November 16, 2022

Abstract

Cadherins are distributed in metazoans. In vertebrates, the most common along with protocadherins are, the classical cadherins, type I and type II. In invertebrates, type III and IV ab cadherins. Here, we focused on the lepidopteran epithelial classical DE-cadherins because they function as a protein-like receptor of *Bacillus thuringiensis* Cry proteins. Alignment and blasting of protein sequences retrieved from NCBI and a phylogenetic tree was built. A common ancestor and several clades were formed among the different lepidopteran cadherins. The most common are *Helicoverpa armigera* and *Spodoptera litura.* Remarkably, lepidopteran cadherins (DE-Cad) from *Spodoptera frugiperda* (n = 20) matched human E-cadherins with an E value of 1.06 e-47 and a bit score of 177). This data indicate that genes that encode the classical cadherins, present in both, the epithelial midgut of lepidopterans (i.e. *Spodoptera frugiperda)* and/or the epithelial in mammals, are homologous (around 50%) in the N-terminal region ectodomains, including the conserved free-linkers calcium binding sites. This could have an impact in the diversity of the functionality of these proteins.

Keywords: Classical Cadherins; Vertebrates; Invertebrates; Arthropods; Lepidopteran; *Bacillus thuringiensis*; Cry Proteins

Introduction

In the insect midgut, the epithelial tissues, represent the dynamic physical and chemical barriers of cells, separating the lumen from the internal [1]. The tight association in the assembly of cellular junctions mechanically stabilizes epithelial tissues. Epithelial cells can change shape or intercalate as tissues deform during morphogenesis [2,3]. During all these events implicated in the epithelial remodeling, signalization pathways that lead to morphogenesis and maintaining the architecture of epithelial tissues [4-6]. In epithelial tissues, one of the major junctional structures is the so called adherens junctions, which in insects are formed by cadherins or cadherin adhesion receptors, specifically tby he classical cadherins, by the catenins (alpha, beta, gamma), the shotgun. The cadherins are required for the tight association between cells, and the association with F-actine allow this. Adherens junction are not static entities, but dynamic activity, which is supported by the interactions among their molecular components and associations as well as by the heterophlic interactions with

other molecules [7-10], thus, being responsible for intercellular adhesion, integrins are for cell-extracellular matrix interactions [7,8] and in the control of structural morphology and functional differentiation [11]. Inappropriate regulation of their expression levels or functionality has been observed in human malignancies leading to aggravated cancer cell invasion and metastasis [12-14].

In general, cadherins are one of the molecular components of tight junctions in bilaterians animals and presumably in non bilaterians animals [11]). The basis structure of the cadherins are formed by the N-terminal region and a C-terminal regions. The structure of the cadherins that shape the adherens junctions is a globular structure similar to the immunoglobulins (IgG) like domain. The N-terminal region are comprised of repeated extracellular domains (ECs) that varied in number in a range of five to thirty four. Each ectodomains is one hundred and ten amino acid residues. Several studies support the role of the N-terminal region extracellular domains in mediating cell-cell homophilic or heterophilic interactions. A C-terminal binding to p120 catenin and beta-catenin/Armadillo at separate sites [12-18]. In invertebrates, classical cadherins may utilize multiple EC domains to form intercellular adhesive bonds [19,20]. The significance of this remains unclear. Invertebrates cadherins apparently underwent less reduction changes in the N–terminal region than vertebrates while maintaining in general the physicochemical and binding sites for intercellular interactions. Specifically, lepidopteran and dipteran cadherins are glycoproteins of around 220 kDa [21,22] immersed in the microvellosity of the insect midgut as transmembranal proteins. The predicted structure of Cry-binding lepidopteran cadherins includes an amino-terminal signal peptide, 8-12 cadherin repeats (CRS), a membrane-proximal extracellular domain (MPED), a transmembrane domain, and a cytoplasmic domains [23]. In the mechanism of interaction of the Cry toxins with cadherins like receptors, binding sites map at or near the membrane-proximal cadherin repeats [24]. Cadherin fragments containing the critical toxin binding region enhance the activities of Cry toxins in lepidopterans and dipterans [25-29]. Furthermore, cadherin gene is essential for *Aedes'* development. Several studies have shown that the cadherin *AaeCad* plays a role in the apical membrane and midgut integrity. Other functions for the dipteran classical cadherins are cell-cell recognition, tissue polarity boundary formation, and coordination of multicellular movement [25,28]. Moreover, homozygous knockout of the cadherin gene in *H. armigera* confers resistance to the Cry1Ac toxin. Cadherin mutants have been identified, and some confer resistance to the Cry toxins in *Heliothis viriscens, Pectinophora gossypiella*, and *Helicoverpa armigera*. Many of these mutations in lepidopterans are null [28,30,31].

Recent studies in the literature have highlighted the phylogenetic relationships among the cadherins present in bilaterians and specifically in hexapods and non hexapods arthropods [23,32]. However, remains to be characterized and pinpoints the phylogenetic relationship among the lepidopteran cadherins, and specifically , with the vertebrate epithelial classical cadherin (E-Cad) (type I). To this end, *in silico* analysis was carried out. This allowed us to assess the homology and the calcium sites at the level of the N-terminal region of the *Spodoptera frugiperda.*

Material and Methods

Alignment of Lepidopteran insect cadherins

Six hundred and forty five protein sequences from insects (*Ostrinus nubilis*, *Bombyx mori*, *Helicoverpa armigera, Spodoptera frugiperda*, *Manduca sexta*) retrieved from NCBI. The Alignments were performed using the program MUSCLE in Unipro U GENE. The phylogenetic tree was built using MEGA 7, PROTEIN BLAST programs (Altachul., *et al*. 1997; Aravind., *et al*. 2001). By another hand, a representative group of type I (E-Cad) vertebrate epithelial (classified as major cadherins) (HUGO Database) (data not shown) were selected to search for homology with lepidopteran cadherins (DE-Cad). The amino sequences ere retrieved also from the NCBI database.

Alignment of lepidopteran classical cadherin (DE-Cad) and vertebrate epithelial classical cadherin (E-Cad)

The alignment between the six hundred forty-five cadherins from Lepidopteran species (*Spodoptera*, *Bombyx*, *Helicoverpa,* and *Manduca*) and vertebrate epithelial classical cadherin (882 aminoacids) were achieved. Seven hundred fifty-five alignments for vertebrate epithelial classical cadherins (E-Cad) (CDH1). A phylogenetic tree was built using MEGA 7, PROTEIN BLAST programs [33,34].

Mapping Calcium interlinkers binding site on lepidopteran classical cadherin (DE-Cad) and vertebrate epithelial classical cadherin (E-Cad)

Seventy-one lepidopterans epithelial cadherin sequences were filtered using the program Web logo3 (71/645 = 11%). Twenty-three sequences best matched with (E-Cad) (CDH1) yielding eighty-two significative alignments. The alignments with the highest score and significant e-value that belong to the

genus *Spodoptera frugiperda* (Evalue, 1e-66) were selected. The number of significative alignments for each sequence (CDH1 versus twenty-three of *Spodoptera frugiperda* cadherin) (Evalue, 1e-66) [33,34] in each position was extracted manually by performing a program with the Lazarus language. Twelve fragments with significative alignments (BLAST) with the vertebrate epithelialc cadherin (E-Cad) Cad) were cut with UGENE. Acidic contact sites (Ca^{2+} binding sites) in human cadherin 1 (CDH1) were identified using PFAM and HUGO and retrieved from the UniProt (https://www.uniprot.org/uniprot/PI2830) and then in epithelial cadherin.

Results

Epithelial classical cadherins from lepidopterans are highly related and have a common ancestor

Lepidopteran amino sequences of the cadherins were retrieved from the NCBI database. An initial blasting and alignment of classical Lepidopteran Cadherins show a common among the three major types of lepidopterans cadherins. A phylogenetic tree of *Manduca (n = 28), Helicoverpa armigera* (n = 2374), and *Spodoptera litura* (n = 1969) cadherins were built (data not shown). A common ancestor, and cluster formation were observed accordingly to the homology among each other. maintained through the different clusters formed. A zoom of this first phylogenetic tree is depicted in figure 1A. A small number of clusters and branching among the three species of lepidopteran cadherins was observed (Figure 1A).

Figure 1A: Phylogenetic relationship of the type IVa (DE-Cad) Lepidopterans cadherins. Non-redundant protein sequences (nr) of type IVa lepidopteran cadherins (DE-Cad) were retrieved from the NCBI database. The Alignment was performed using the Program MUSCLE in Unipro UGENE. The phylogenetic tree was constructed using MEGA 7, PROTEIN BLAST programs (Altachul., et al. 1997; Aravind., et al. 2001). DE-cadherins are clustered in different clades. However, they converge in one common ancestor (A). Two thousand and three hundred seventy-four sequences from *Helicoverpa armiguera*; one thousand and nine hundred eighty-nine from *Spodoptera litura* (black), and twenty-eight from Manduca sexta (supplementary mat). To further analyze these phylogenetic relationship, a second tree ws built using less number of cadherins sequences from Helicoverpa armiguera (blue) (n = 35); Manduca sexta (red) (n = 17) and *Spodoptera frugiperda* (black) (n = 39); *Spodoptera litura* (black) (n = 25). A similar result was obtained (2A). Despite that these cadherins are in different clades, they still converge in one common ancestor. Usually, the cluster of *Spodoptera frugiperda* and *Spodoptera litura* are in the middle of the cluster of cadherins of *Helicoverpa armiguera* and Manduca *sexta*. The lepidopteran protocadherins show a similar pattern of clustering and homology than human protocadherins.

Lepidopteran epithelial classical adherins are close related to the vertebrate epithelial classical cadherin (CDH1)

Six hundred and forty five cadherins sequences of different lepidopteran organisms were aligned and blasted with human epithelial cadherins isoform 1 (CDH1, 882 amino acids) (PIFAM, HUGO) [33,34]. A common ancestor and branching was observed suggesting a close relationship between both types of cadherins (data not shown). A zoom of one of these regions (Figure 1B) show that both types of cadherins are related, CDH1 form a close cluster that some of the cadherins of lepidopteran species.

Figure 1B: Phylogenetic relationships among lepidopteran cadherins and vertebrate epithelia classical cadherin. To determine the homology between both types cadherins, type I (E-Cad) (CDH1) and the type IVa (DE-Cad), a phylogenetic tree was built, respectively. Sequences for type I (E-Cad) (CDH1 = 882 a.a; and the CDH2 = 822 a.a.), and the type IVa lepidopterans cadherins (n = 645). The alignment was made with the UNIPRO UGENE program of NCBI (supplementary mat). The tree was built using the evolutive model Neighbor-Joining program (suppl mat). A common ancestor is observed. However, due to the high number of sequences, and in order to visualize more precisely the cluster formed, a second tree was built using the three most common species of type IVa lepidopterancadherins. Thus, we observed several clusters, formed among the different type IVa cadherins that are close to type I (E-Cad) (CDH1) and the common ancestor (2B). The clade formed by some of the *Helicoverpa* cadherins are far from the CDH1.

Spodoptera frugiperda Epithelial classical adherins are homologous at the N-terminal region with the vertebrate epithelial classical cadherin (CDH1)

From the seven hundred and fifty nine alignments between type I (E-Cad) (CDH1) and the six hundred forty five lepidopteran cadherins equences, seventy-one cadherin sequences belonged to *the Spodoptera* genus, around 11% of the total cadherins were recovered (Suppl. Mat I). To perform a more astringent analysis in the search for the region of highest homology between vertebrate epithelial cadherin (E-Cad), and lepidopteran classical cadherin (DE-Cad), twenty-three type IVa (DE-Cad) of *Spodoptera frugiperda* (statistic E value closer to cero (1e-47 and 1e-46) (suppl mat II) were blasted with CDH1 (Figure 2A-B) We found regions in the sequence with high amino acid conservation showed in bars (upper side of the sequence alignment) and of high homology on the extracellular domains of both types of cadherins, between 126-183 (EC1); 21-252 (EC2); 445-489 (EC3); 455.604 (EC4) (Figure 2A-B). Among the most conserved amino acid residues D, E, F, Y, G, P, V, L [35].

Furthermore, using PFAM (Finn., *et al*. 2014) and Uniprot UGENE. Remarkably, binding Ca^{2+} sites sequences (DXD, DYNDN) were found in *Spodoptera frugiperda* cadherin and mapped in the same acidic amino acids than type I (E-Cad) (CDH1) (Figure 2C) Four Ca^{2+} sites were predicted in positions EC1 (131-133); EC2 (382-384); EC3 (394-396); EC4 (545-548) (Figure 2B upper and lower pannel).

Discussion and Conclusion

In silico analysis of the lepidopteran cadherins allowed us to assess the homology between *Spodoptera frugiperda* epithelial classical cadherin and the vertebrate epithelial classical cadherins at the level of the N-terminal and the calcium interlinkers binding sites. Altogether the data show that despite the homology between both types of cadherins, host interspecies are important and might be implicated in the diversity of the functionality of these proteins.

Figure 2A

Figure 2B

Figure 2B

Figure 2: Homology between lepidoptera classical cadherins and vertebrate epithelia classical cadherin. Seventy one most significant alignments were recovered from the alignment of the type I (E-Cad) (CDH1) and the type IVa (DE-Cad) (n = 645) (suppl mat) (I). The alignments with the highest score and significant e-value belonged to the genus *Spodoptera frugiperda* (E value, 1e-66) (Suppl mat) (II). Twenty three cadherin sequences had it this value and they were aligned with type I (E-Cad) (CDH1). From this, a final filtering selection were made and twelve fragments from the *Spodoptera frugiperda* were finally aligned with type I (E-Cad) (CDH1) (A-B). In the alignment of CDH1 versus the 23 cadherin sequences of *Spodoptera frugiperda* is shown the underlined the EC1-EC4 ectodomains that map in the N-terminal region of the cadherins (A-B). In color are the conserved residues of aminoacids between the CDH-1 and DE-Cad of S. *frugiperda*. It can distinguished in colors the aminoacid residues conserved in the ectodomains (EC1-EC5) that form part of the N-terminal region of both types of cadherins. Furthermore, in (C), in the upper panel, are the general structure of the type I (E-Cad) and the type IVa (DE-Cad) cdherins. The Extracellular cadherin-like repeats (EC1-5); the transmembrane domain (TM); the short, 17 aa-long fragments between the transmembrane and the P120-binding domains. In the lower panel, the blue square is the alignment but with the predicted acidic contact sites (Ca^{2+} binding sites) mapped in the same positions in either vertebrate (type I-II) (E-cad) (CDH1) and the type IVa (DE-Cad) lepidopteran classical (*Spodoptera frugiperda*) cadherin identified using PFAM [44].

In general, cadherin plays an essential role in cell recognition, adhesion activities, and morphogenesis [7,15]. These proteins function in cell-cell contact in tissues and participate actively in homophilic and heterophilic interactions inter and intracellular adhesion binding, triggering thus a cascade of signalization that leads to development and morphogenesis [22,23,36,37]. It is well accepted and demonstrated especially in vertebrates, that the functionality of the cadherins reside in both regions. N-terminal

and C-terminal region. Adhesion properties of cadherin are through the extracellular domains (EC) and the calcium-binding sites of the N-terminal region, that play a critical role in keeping the extracellular domains and mediating binding between cadherins proteins on opposing cells. Aspartate amino acid residues coordinate calcium ions at the base for the cadherin extracellular domains. These domains are the most conserved across different species [35] while in the C-terminal region are the cytosolic tail, involved in intracellular signaling and interactions with p120 catenins, β-catenin, α-catenin, and indirectly, with the cytoskeleton (F-actin) [25,38]. The role of these organization in the either the N-terminal or C-terminal regions have been shown through mutations in human classical cadherins mapped in the ECs led to effects in growth and proliferation [14]. While, mutagenesis in Lepidopteran DE-Cadh increases insect resistance [25,27,31,32,39].

The evolution in metazoans, vertebrates and invertebrates have dictated that a set of proteins (cadherins, actin, integrins, cytoskeleton) can shape the architecture of tissues. These components are essential components of the tight junction in epithelial cells in mammals and in adherent junctions in insects [1,12,14,23,32,38-40]. Vertebrate epithelial classical cadherin (E-cadherin) (type I) participate as receptor for the entry of *Listeria monocytogenes* [41], as receptor for a pneumococcal surface antigen [42], and as receptor for AlS3 of *Candida albicans* for endocytosis [43]. In invertebrates, specifically in some orders of insects, epithelial classical cadherins (DE-Cad) function as a receptor of Bt Cry proteins [21,25].

In arthropods usually classical cadherins are found in epithelial and non epithelial tissues. In the first case are represented by type IVa cadherins, and are usually found in insects, crustacean and non-insects hexapods. In the second case are commonly represented by the neural cadherins present in *Drosophila melanogaster*, type III cadherins, The evolutionary origin of invertebrates classical cadherins, type IV has been suggested are derived paralogs of type III cadherins. The original ancestor possess 17 or a higher number of ECs. The reduction mechanism process in the N-terminal lead to the diversity of classical cadherins in structure, domain organization and functionality [23]. However, the five ectodomains in the N-terminal region remains conserved through the evolution process as strategy for homophilic interactions. The common ancestral type III classical cadherins present in the bilaterians, vertebrates, hexapods, brachiopods, and arthropods gave rise to the type IV cadherins, with a varied number of extracellular domains as an strategy for heterophilic interactions [29,30,41-43], The deduced epithelial classical cadherin from *Spodoptera frugiperda,* yields a transmembrane protein with an apparent molecular mass of 220 kDa. Furthermore, aminoacid sequence alignment of the lepidopteran cadherins with the human classical cadherins show homology (around 50%) (Figures 1B; 2A-B) (Oda and Takeishi., 2011; Sasaki., *et al.* 2017) [4,23] at the level of the N-terminal region (ectodomains) and the calcium binding sites [44] (Figure 2C), despite that the second ones are shorter in length. The alignment with the highest score and Evalue was for human E-cadherin Isoform 1 (CDH1) and DE-cad from *Spodoptera frugiperda*, one of the most representative Lepidotperan, target of the Bt Cry proteins. Altogether the data obtained from the alignment of both cadherins suggest that they are forming a close clade, and they are conserved in acidic residues that form part of the four binding sites to calcium such as DRE (75-77; 335-337 and 545-547), NDN (131-133; 382-384; 600-602), DXD (X = any aminoacid) (446-448; 568-570), that aligned and machete well with those of the human classical cadherin (CDH1). Other highly conserved aminoacids like phenyl alanine (56F), Glycine (65G); Tyrosine (87Y), Valine (V), Leucine (89L) in the extracellular domains or ectodomains (EC1-EC5) (Figure 2A-B).

In summary, referring specifically to the classical cadherin of *Spodoptera frugiperda,* the most homology with the epithelial classical cadherins in mammals reside in the extracellular ectodomains of the N-terminal region and the free interlinkers calcium binding sites. Altogether the data show that despite the homology between both types of cadherins, host interspecies might playing a key role in the diversity of the functionality of these proteins.

Disclosure Statement

The authors declare “not a conflict of interest”.

Author’s Contribution

G.G.G.M. conceptualization, design and written of the paper. A.M.O. J.M.D.P; S.V.C.J. G.E.O.R. bioninformatic, informatics work.

Data Availability Statement

Gabriela Edith Olguin-Ruiz and Carlos Javier Sanchez-Vallejo are employed by the E.N.C.B. IPN, CD MEX. Juan Manuel Perez Diaz is employed by the University Autonome of Zacatecas. Arnoldo Mendoza-Osorno works for a company in CDMX. Still undergraduate. There are no patents, products in development, or marketed products to declare. This does not alter the author’s adherence to all the policies on sharing data and materials.

Financial Statement

The study did not receive funding from any dependence nor a grant for the present study work. However, G.G.G.M received a fellowship from PERFIL PRODEP (Federal Program of the National Secretary of Education-SEP. MX) and SNI CONACYT.

Acknowledgments

Authors are grateful for colleagues' support.

Bibliography

1. Caccia S., *et al*. "The amazing complexity of insect midgut cells: types, pecularities, and functions". *Cell Tissue Research* 377 (2019): 505-525.

2. Guillot C and Lecuit T. "Mechanics of epithelial tissue homeostasis and morphogenesis". *Science* 340 (2013): 1185-1189.

3. Fahey B and Degnan BM. "Origin of animal epithelia: insight from the sponge genome". *Evolution and Development* 1 (2010): 2601-2617.

4. Oda H and Takeichi M. "Structural and functional diversity of cadherin at the adherens junction". *Journal of Cell Biology* 193 (2011): 1137-1146.

5. Harris TJC and Tepass U. "Adherens junctions: from molecules to morphogenesis". *Nature Reviews Molecular Cell Biology* 11 (2010): 502-514.

6. Oda H., *et al*. "Diversification of epithelial adherens junctions with independent reductive changes in cadherin form: identification of potential molecular synapomorphies among bilaterians". *Evolution and Development* 7 (2005): 376-389.

7. Takeich M. "Dynamics contacts: rearranging adherens junctions to drive epithelial remodeling". *Nature Reviews Molecular Cell Biology* 15 (2014): 397-410.

8. Klezovitch O and Vasioukhin V. "Cadherin, signaling: keeping cells in touch". *F1000 Research* 4 (2015): 550.

9. Leckband D and Prakasam A. "Mechanism and dynamics of cadherin adhesion". *Annual Review of Biomedical Engineering* 8 (2006): 259-287.

10. Yonemura S. "Cadherin-actin interactions at adherens junctions". *Current Opinion in Cell Biology* 23 (2011): 515-522.

11. Jaiganesh A., *et al*. "Beyond Cell-Cell Adhesion: Sensational Cadherins for Hearing and Balance". *Cold Spring Harbor Perspectives in Biology* 4.10 (2018): a029280.

12. Lecuit T and Yap AS. "E-cadherin junctions as active mechanical integrators in tissue dynamics". *Nature Cell Biology* 17 (2015): 533-539.

13. Hong S., *et al*. "Cadherin exits the junction by switching its adhesive bond". *The Journal of Cell Biology* 192 (2011): 1073-1083.

14. Vizirianakis JS., *et al*. "Dominant-negative E-cadherin alters adhesion and reverses contact inhibition of growth in breast carcinoma cells". *International Journal of Oncology* 21 (2002): 135-144.

15. Kaderbatcha ADM., *et al*. "Extracellular domains of E-cadherin determine key mechanical phenotypes of an epithelium through cell- and non-cell-autonomous outside-in signaling". *PLoS One* 16 (2021): e0260593.

16. Kim NG., *et al*. "E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components". *Proceedings of the National Academy of Sciences of the United States of America* 108 (2011): 11930-11935.

17. Nichols SA., *et al*. "Origin of metazoan cadherin diversity and the antiquity of the classical cadherin/β-catenin complex". *Proceedings of the National Academy of Sciences of the United States of America* 109 (2012): 13046-13051.

18. Gul IS., *et al*. "Evolution and diversity of cadherins and catenins". *Experimental Cell Research* 358 (2017): 3-9.

19. Hsu SN., *et al*. "Conserved alternative splicing and expression patterns of arthropod N-cadherin". *PLoS Genetics* 5 (2009): e1000441.

20. Yanekura S., *et al*. "Adhesive but not signaling activity of *Drosophila* N-cadherin is essential for target selection of photoreceptor afferents". *Developmental Biology* 304 (2007): 759-770.

21. Fabrick J., *et al*. "A novel *Tenebrio molitor* cadherin is a functional receptor for *Bacillus thuringiensis* Cry3A toxin". *Journal of Biological Chemistry* 284 (2009): 18401-18410.

22. Zhang H., *et al*. "Intra-and-extracellular domains of the *Helicoverpa armiguera* cadherin mediate Cry1Ac cytotoxicity". *Insect Biochemistry and Molecular Biology* 86 (2017): 41-49.

23. Sasaki M., *et al*. "Evolutionary origin of type IV classical cadherins in arthropods". *BMC Evolutionary Biology* 17 (2017): 142-165.

24. Hara H., *et al*. "A cadherin-like protein functions as a receptor for *Bacillus thuringiensis* Cry1Aa and Cry1Ac toxins on midgut epithelial cells of *Bombyx mori* larvae". *FEBS Letter* 538 (2003): 29-34.

25. Chen J., *et al*. "Aedes cadherin receptor that mediates *Bacillus thuringiensis* Cry11A toxicity is essential for mosquito development". *PLOS Neglected Tropical Diseases* 14 (2020): e0007948.

26. Lu Q., *et al*. "A fragment of cadherin-like protein enhances *Bacillus thuringiensis* Cry1B and Cry1C toxicity to *Spodoptera exigua* (Lepidoptera: Noctuidae)". *Journal of Integrative Agriculture* 11 (2012): 628-638.

27. Contreras E., *et al*. "Sodium solute symporter and cadherin proteins act as *Bacillus thuringiensis* Cry3Ba toxin functional receptor in *Tribolium castancum"*. *Journal of Biological Chemistry* 288 (2013): 18013-18021.

28. Pigott CR and Ellar DJ. "Role of receptors in *Bacillus thuringiensis* crystal toxin activity". *Microbiology and Molecular Biology Reviews* 71 (2007): 255-281.

29. Adang MJ., *et al*. "Diversity of *Bacillus thuringiensis* crystal toxins and mechanism of action". *Advances in Insect Physiology* 47 (2014): 39-87.

30. Jurat-Fuentes JL., *et al*. "The HevCaLP protein mediates binding specificity of the Cry1A class of *Bacillus thuringiensis* toxins in *Heliothis virescens"*. *Biochemistry* 43 (2004): 14299-14305.

31. Xu X., *et al*. "Disruption of a cadherin gene associated with resistance to Cry1Ac delta-endoxtin of *Bacillus thuringiensis* in *Helicoverpa armiguera". Applied and Environmental Microbiology* 71 (2005): 946-954.

32. Nishiguchi S., *et al*. "Divergence of structural strategies for homophilic E-cadherin binding among bilaterians". *Journal of Cell Science* 129 (2016): 3309-3319.

33. Altachul SF., *et al*. "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs". *Nucleic Acids Research* 25 (1997): 3389-3402.

34. Aravind Madden TI., *et al*. "Improving the accuracy of PSI-BLAST protein datrabase searches with composition-based statistics and other refinements". *Nucleic Acids Research* 29 (2001): 2994-3005.

35. Prakasam A., *et al*. "Calcium site mutations in cadherin: impact on adhesion and evidence of cooperativity". *Biochemistry* 45 (2006): 6930-6939.

36. Mountoufaris G., *et al*. "Reading, and Translating the Clustered Protocadherin Cell Surface Recognition Code for Neural Circuit Assembly". *Annual Review of Cell and Developmental Biology* 34 (2018): 471-493.

37. Chen WV and Maniatis T. "Clustered protocadherins". *Development* 140 (2013): 3297-3302.

38. Weisherg VCH and Maniatis T. "Clustered Protocadherins". *Development* 140 (2013): 3297-32302.

39. Du L., *et al*. "Cadherin CsCad plays differential functional roles in Cry1Ab and Cry1C intoxication in *Chilo suppressalis". Science Reports* 9 (2019): 8507-8522.

40. Zhao J., *et al*. "Diverse cadherin mutations conferring resistance to *Bacillus thuringiensis* toxin Cry1Ac in *Helicoverpa armigera". Insect Biochemistry and Molecular Biology* 40 (2010): 113-118.

41. Mengoud J., *et al*. "E-cadherin is the receptor for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells". *Cell* 22.84 (1996): 923-932.

42. Anderton JM., *et al*. "E-cadherin is a receptor for the common protein pneumococcal surface antigens A (PlsA) of *Streptoccous pneumonia". Microbial Pathogenesis* 42 (2007): 225-236.

43. Phan QT., *et al*. "Als3 is a *Candida albicans* invasion that binds to cadherins and induces endocytosis by host cells". *PLoS Biology* 5 (20017): e64.

44. Finn RD., *et al*. "Pfam: the protein families database". *Nucleic Acids Research* 42 (2014): D222-D230.