Research Articles

Preliminary Report: Homology Modeling of Human, Dog and Catβ-adrenergic Receptors

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Abstract

Beta 2-adrenergic Receptors are widely distributed in heart, airway smooth muscle, liver, skeletal muscle and adipose tissue. The specific ligand, beta 2-agonist, binds at the specific binding site on ADRB2 then the signal transduction pathways begins. The three-dimensional configuration of ADRB2 is uniquely and determines by its amino acid sequence. In this study, human ADRB2, dog ADRB2, and cat ADRB2 sequences were studied by sequence of P07550, P54833 and Q9TST5, respectively. The similarity of human ADRB2 and dog ADRB2 was 90% whereas the similarity between human ADRB2 and cat ADRB2 was 89%. In addition, the similarity between dog ADRB2 and cat ADRB2 was 95%. The *in silico* ADRB2 models were generated and compared among species of interest. The results show that the shapes and the pocket sites of ADRB2 differ between species. The differences of ADRB2 three-dimensional configuration may give explanations about the variety of interaction between ADRB2 and its agonist among human, dog and cat. Further study of protein-protein interaction, structure-based drug design, and novel function of ADRB2 in companion animals may have basis from human ADRB2.

Keywords: ADRB2, Homology modeling, *in silico*, Human, Dog, Cat

การทดลองเบื้องต้น: การสร้างแบบจำลองของตัวรับอะดรีเนอร์จิกชนิดเบต้าในมนุษย์ สุนัข และแมวโดยใช้เทคนิค Homology Modeling

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บทคัดย่อ

ตัวรับอะดรีเนอร์จิกชนิดเบต้าทูถูกพบว่ามีการกระจายตัวอยู่ในหัวใจ กล้ามเนื้อเรียบในทางเดินหายใจ ตับ กล้ามเนื้อลาย และเนื้อเยื่อไขมัน เบต้าทูอะโกนิสต์ซึ่งเป็นลิแกนด์ที่จำเพาะเจาะจงต่อตัวรับชนิดนี้จะเข้าจับตรงตำแหน่งที่เฉพาะเจาะจงบนตัวรับอะดรีเนอร์จิกชนิดเบต้าทู (ADRB2) แล้วกระตุ้นให้เกิดวิถีนำส่งสัญญาณ โครงสร้างสามมิติของ ADRB2 มีลักษณะเฉพาะและถูกกำหนดโดยลำดับกรดอะมิโน การศึกษาครั้งนี้ใช้ลำดับกรดอะมิโน P07550, P54833, Q9TST5 สำหรับศึกษา ADRB2 ในมนุษย์ สุนัข และแมวตามลำดับจากผลการศึกษาพบว่าลำดับกรดอะมิโนของ ADRB2 ในมนุษย์และสุนัขมีความคล้ายคลึงกัน 90 เปอร์เซ็นต์ ในขณะที่ ADRB2 ในมนุษย์และแมวมีความคล้ายคลึงกัน 89 เปอร์เซ็นต์ นอกจากนี้ยังพบว่า ADRB2 ในสุนัขและแมวมีความคล้ายคลึงกันถึง 95 เปอร์เซ็นต์ในการวิจัยครั้งนี้ได้ศึกษาแบบจำลองของ ADRB2 โดยใช้ ***in silico*** **models** ซึ่งทำการจำลองโครงสร้างและเปรียบเทียบความแตกต่างระหว่างมนุษย์และสัตว์ที่สนใจศึกษา จากผลการจำลองโครงสร้างพบว่า ADRB2 ในมนุษย์และสัตว์มีความแตกต่างกัน ทั้งในเรื่องของโครงสร้างทุติยภูมิและตำแหน่งที่คาดว่าลิแกนด์จะเข้าทำปฏิกิริยา อนึ่ง โครงสร้างสามมิติของ ADRB2 อาจจะช่วยในการอธิบายถึงความหลากหลายของการเข้าทำปฏิกิริยาระหว่าง ADRB2 และลิแกนด์ในมนุษย์ สุนัข และแมว ผลการศึกษาโดยใช้แบบจำลองของ ADRB2 ในมนุษย์จะใช้เป็นข้อมูลพื้นฐานในการศึกษาปฏิสัมพันธ์ระหว่างโปรตีน การออกแบบยาโดยอาศัยแบบจำลองโครงสร้าง และการค้นพบหน้าที่ใหม่ๆ ของ ADRB2 ในสัตว์ชนิดต่างๆ ต่อไป

คำสำคัญ : ตัวรับอะดรีเนอร์จิกชนิดเบต้าทู Homology modeling *in silico*, มนุษย์ สุนัข แมว

Introduction

Beta-adrenergic Receptor (ADRB) is a seven transmembranefamily of protein, encoded by a gene on the long arm of chromosome 5 (Johnson, 1998).This receptor has been subdivided into 3 groups; beta-1, beta-2 and beta-3.Beta 2-adrenergic Receptors (ADRB2) are widely distributed in heart, airway smooth muscle, liver, skeletal muscle and adipose tissue (McNeel, and Mersmann, 1999). The specific ligand, beta 2-agonist, binds at the specific binding site on ADRB2 then the signal transduction pathways begins (Johnson, 1998).

It is probably that beta 2-agonistsbind to the specific ADRB2 and temporary stabilizing ADRB2 in their activated state. The beta 2-agonist may affect shifting of the equilibrium, not through induces a conformational changes but through ADRB2-beta 2-agonist complex itself (Ahrens and Smith, 1984). Cryo-EMand three dimensional studies are the latest techniques to solve and to simulate the ADRB2-beta 2-agonist complex. Therefore, the valuable information about the ADRB2, the beta 2-agonist and the ADRB2-beta 2-agonist complex are rising after the post genomic era.

Homology modelingtechnique is the technique that predicts a structure from its sequence with an accuracy which is comparable to the best results achieved experimentally.During evolution, the protein structure is more stable and changes much slower than its associated sequence. Theoretically, the similar sequences adopt practically identical structures and distantly related sequences still fold into similar structures. In addition, homology modeling is the only one technique that can obtain information if other experimntal fail (Krieger, Nabuurs, and Vriend, 2003).

This study compared ADRB2 sequences among 3 species; *Homo sapiens, Canis lupus familiaris,* and *Feliscatus*. We aimed to simulate three dimensional structures of ADRB2 by homology modeling technique.

Materials and Methods

Sequences and structures

Amino acid sequences of human, dog and cat ADRB2 were collected from Protein Data Bank (PDB). Human ADRB2, dog ADRB2, and cat ADRB2 sequence were downloaded from UniProt (http://www.uniprot.org) by sequence of P07550, P54833 and Q9TST5, respectively. Structural data for ADRB2 was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB). Human ADRB2-Gs Protein complex was selected to be a role model of this study (RCSB-PDB ID number 3sn6).

Sequences and structural comparisons

Sequences of human ADRB2 were extracted from the RCSB-PDB file information then compared with that of dog ADRB2 and cat ADRB2 using the multiple sequences alignment. The similarities of ADRB2 sequences between human, dog and cat were compared and pairwise sequences alignment operations by Clustal O (http://www.clustal.org/omega/) which is the latest addition to the Clustal family.

Homology model construction

Coordinate files were assigned to the ligand binding domain of human ADRB2, dog ADRB2 and cat ADRB2 from the regions of greatest structural conservation among the ADRB2 structures. The next step was close comparison with available structures and constructed the three dimensional structures of particular ADRB2.

Results

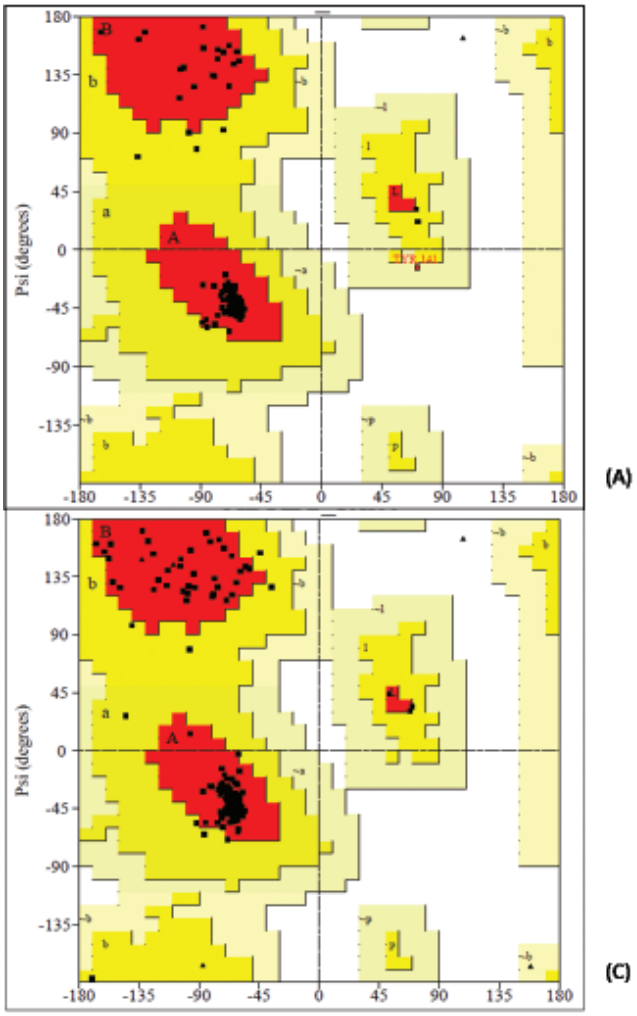
Table 1 Scores table from CLUSTAL O (1.1.0) multiple sequences alignment of human ADRB2, dog ADRB2 and cat ADRB2.

Species	Length (AA)	Species	Length (AA)	Score
Homo sapiens	413	*Canis lupus familiaris*	415	90.0
Homo sapiens	413	*Feliscatus*	418	89.0
Canis lupus familiaris	415	*Feliscatus*	418	95.0

Differences of amino acid sequences between human ADRB2, dog ADRB2 and cat ADRB2 were compared and demonstrated in 3 categories; identical (*), conserved (:), semi-conserved (.).

Figure 1 CLUSTAL O(1.1.0) multiple sequences alignment of human ADRB2, dog ADRB2 and cat ADRB2.

Whenever the tertiary structure of a native protein was studied, the varieties of the rotatable bonds were plotted. Ramachandran plot represented the relationship between Phi (ϕ) and Psi (ψ) angles; however, the steric effect influenced the angles of Phi and Psi. The complexities of the rotatable bonds made the specific pattern of Ramachandran plot.



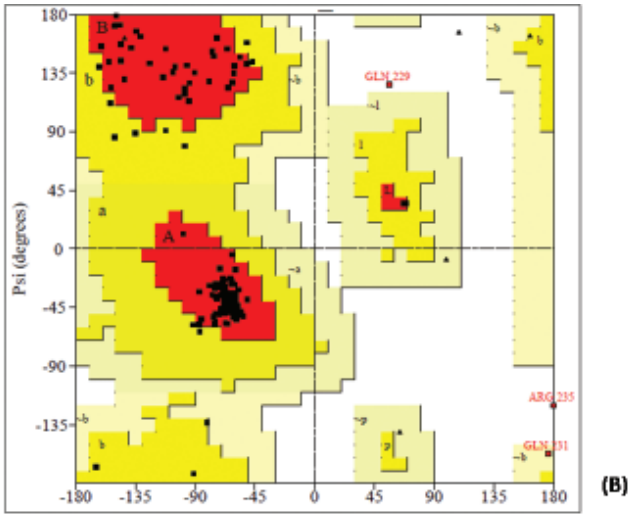


Figure 2 Ramachandran's plot of human ADRB2 (A), dog ADRB2 (B) and cat ADRB2 (C) were reported at 96.5, 94.5 and 97.3% respectively.

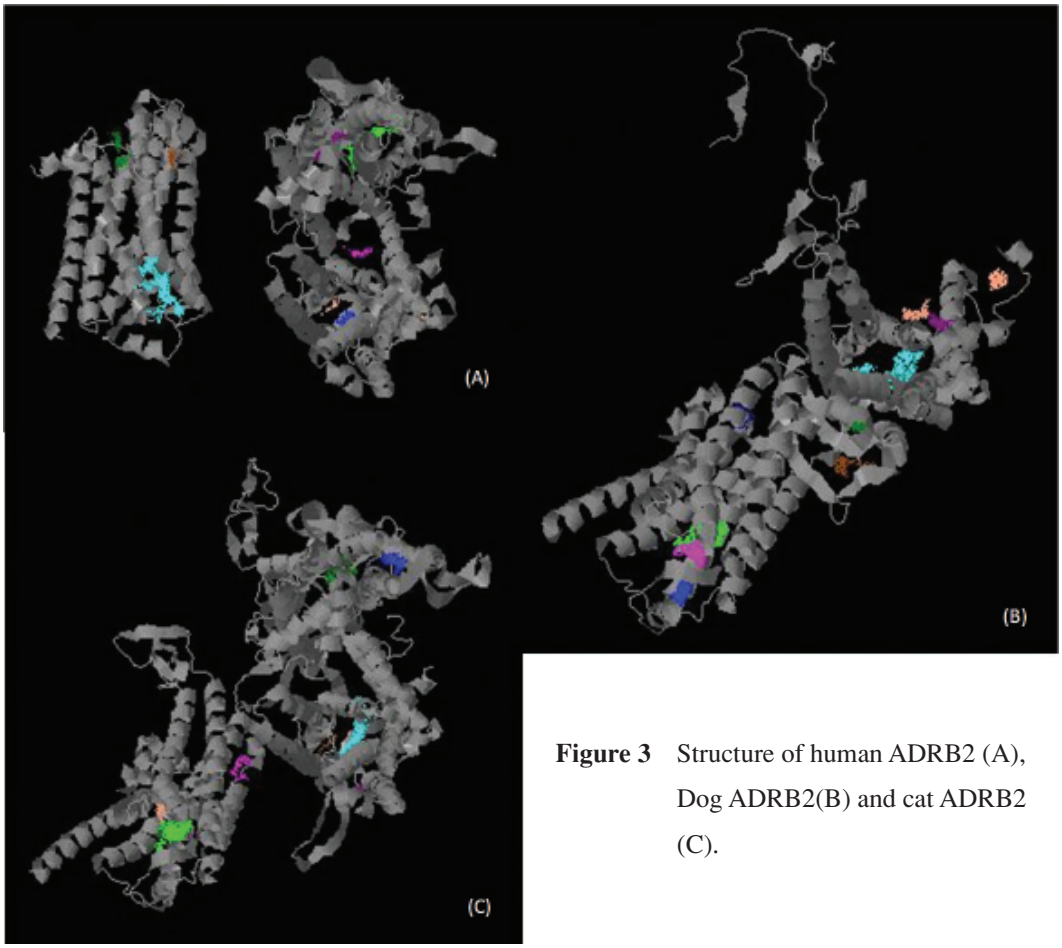


Figure 3 Structure of human ADRB2 (A), Dog ADRB2(B) and cat ADRB2 (C).

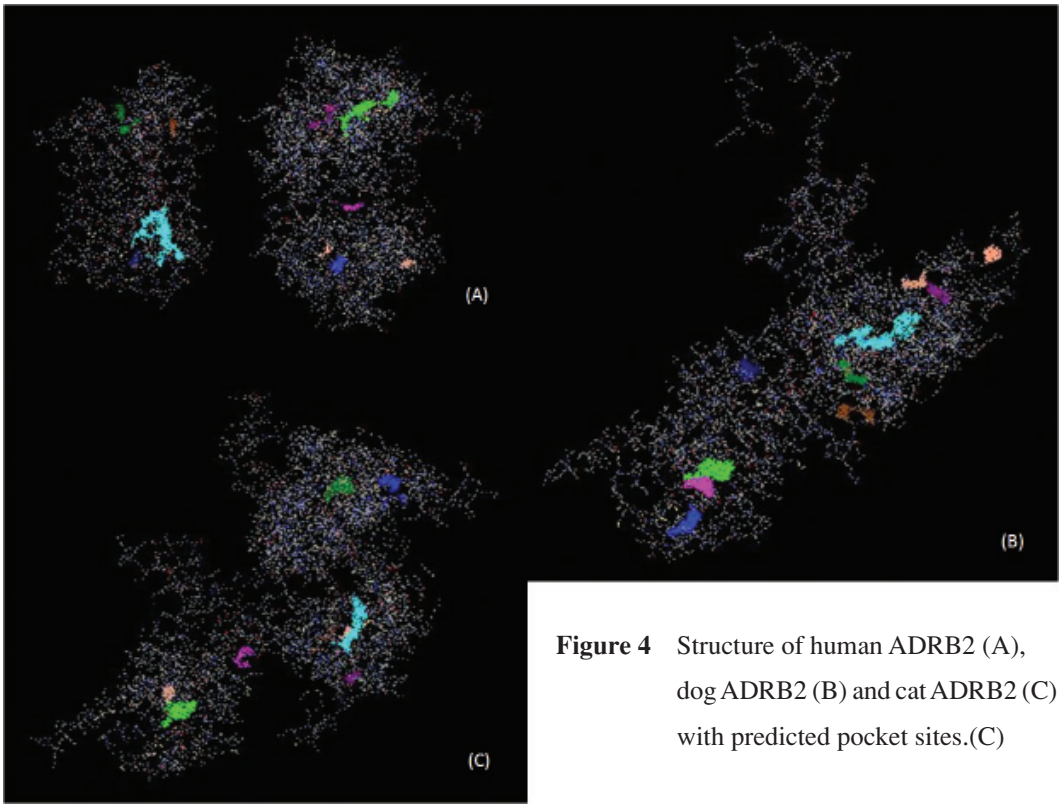


Figure 4 Structure of human ADRB2 (A), dog ADRB2 (B) and cat ADRB2 (C) with predicted pocket sites.(C)

Discussion

Post-genomic era provides the valuable information of protein structure and function. New genes and structures can provide bridges between superfamilies which previously showed little evidence of homology (Todd, Orengo, and Thornton, 2001). Figure 2 shows the residues in most favored regions from Ramachandran plot of human ADRB2, dog ADRB2, and cat ADRB2 which are 96.5%, 94.5% and 97.3%, respectively. The three-dimensional configuration of a protein is uniquely and determines by its amino acid sequence (Epstain, Goldberger, and Anfinsen, 1963); however, we can only underestimate the functional variation observed especially, in the midnight zone of sequence and structural similarity (Todd, Orengo, and Thornton, 2001).

Further study of protein-protein interaction, structure-based drug design, and novel function of ADRB2 in companion animals may have basis from human ADRB2.

Conclusion

The present study has demonstrated that the amino acid sequences of ADRB2 are very similar among species of interest. Interestingly, human ADRB2, dog ADRB2, and cat ADRB2 showedstructural similarity but the different in predicted pocket sites had been observed. The differences of ADRB2 three-dimensional configuration may give an explanation about the variety of interaction between ADRB2 and its agonist among human, dog and cat.

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References

Ahrens R.C., and Smith G.D. 1984. Albuterol: an adrenergic agent for use in the treatment of asthma; Pharmacology, pharmacogenetics and clinical use. Pharmacology. 4 (3): 105-121.

Awede B.L., Thissen J.-P. andLebacq J. 2002. Role of IGF-I and IGFBPs in the changes of mass and phenotype induced in rat soleus muscle by clenbuterol. American Journal of Physiology-Endocrinology and Metabolism. 282: E31-E37.

Bodine S.C., Stitt T.N., Gonzalez M., Kline W.O., Stover G.L., Bauerlein R., Zlotchenko E., Scrimgeour A., Lawrence J.C., Glass D.J. and Yancopoulos G.D. 2001. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and canprevent muscle atrophy *in vivo*. Nature Cell Biology. 3: 1014-1019.

Brown D., Sinha-Hikim A.P., Kovacheva E.L. and Sinha-Hikim I. 2009.Mouse model of testosterone-induced muscle fiber hypertrophy involvement of p38 mitogen-activated protein kinase-mediated Notch signaling.Journal of Endocrinology. 201: 129-139.

DeLisle R.K., Yu S.J., Nair A.C. and Welsh W.J. 2001. Homology modeling of the estrogen receptor subtype beta (ER-beta) and calculation of ligand binding affinities.Journal of Molecular Graphics andModelling. 20(2): 155-167.

Epstain C.J., Goldberger R.F., and Anfinsen C.B. 1963. The genetic control of tertiary protein structure. Model systems.Cold Spring Harbor Symposia on Quantitative Biology. 28: 439-449.

Favero M., Jiang D-J., Chiamuler C., Cangiano A. and Fumagalli G.F. 2008. Expression of small-conductance calcium-activated potassium channels (SK3) in skeletal muscle: regulation by muscle activity. Journal of Physiology. 586.19: 4763-4774.

Goodman C.A., Mabrey D.M., Frey J.W., Miu M.H., Schmidt E.K., Pierre P., and Hornberger T.A. 2011.Novel insights into the regulation of skeletal muscle protein synthesis as revealed by a new nonradioactive in vivo technique.The FASEB Journal. 25: 1028-1039.

Johnson M. 1998. The β-Adrenoceptor. American Journal of Respiratory and Critical Care Medicine. 158: S146-S153.

Krieger E., Nabuurs S.B., and Vriend G. 2003. "Homology Modeling" in Structural Bioinformatics (Editors, Borne, P.E. & Weissig H.). Wiley-Liss, Inc. 507-521.

McNeel R.L., and Mersmann H.J. 1999. Distribution and quantification of beta1-, beta2-, and beta3-adrenergic receptor subtype transcripts in porcine tissues. Journal of Animal Science. 77: 611-621.

Musa M., Fernando S.M., Chatterjee D. and Monks D.A. 2011.Subcellular effects of myocyte-specific androgen receptor over expression in mice.Journal of Endocrinology. 210: 93-104.

Sandri M. 2008. Signaling in muscle Atrophy and Hypertrophy. Physiology. 23(3): 160-170.

Sinha-Hikim I., Artaza J., Woodhouse L., Gonzalez-Cadavid N., Singh A.B., Lee M.I., Storer T.W., Casaburi R., Shen R. and Bhasin S. 2002. Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. American Journal of Physiology-Endocrinology and Metabolism. 283:E154-E164.

Sinha-Hikim I., Roth S.M., Lee M.I. and Bhasin S. 2003.Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men. American Journal of Physiology-Endocrinology and Metabolism. 285: E197-E205.

Todd A.E., Orengo C.A. and Thornton J.M. 2001. Evolution of function in protein superfamilies, from a structural perspective.Journal of Molecular Biology. 307: 1113-1143.

Wessel T. Van, De Haan A., Van Der Laarse W.J., Jaspers R.T. 2010. The muscle fiber type-fiber size paradox: hypertrophy or oxidative metabolism? European Journal of Applied Physiology. 110(4): 665-694.

Woodhouse L.J., Reisz-Porszasz S., Javanbakht M., Storer T.W., Lee M., Zerounian H. and Bhasin S. 2003. Development of models to predict anabolic response to testosterone administration in healthy young men.American Journal of Physiology-Endocrinology and Metabolism. 284: E1009-E1017.