Polymorphism of follicle stimulating hormone receptor influences the 3D structure and its binding pattern to FSH in Bos taurus

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ABSTRACT

Follicle stimulating hormone (FSH) bond with the extracellular domain of the FSH receptor (FSHR) stimulates a cascade of the intracellular process that leads to folliculogenesis. This study aimed to elucidate the effects of FSHR polymorphism of its structure and function on Bos taurus through computational technology. The FSHR sequences were retrieved from Genbank. The polymorphism was identified using alignment analysis and the 3D structure of the FSHR was done by Swiss models. Results showed that FSHR of Bos taurus has three polymorphisms that located at amino acid residues 18th to 259th. The polymorphisms may alter its ability to bind with FSH. Molecular docking analysis indicated that all variant of FSHR potentially changes the pattern and affinity binding into FSH that may have an impact on reproduction status of Bos taurus. The study is a warrant for further investigation to explore biomarker of cattle reproduction status based on FSHR gene.

Key words: Amino acid, Bos Taurus, FSHR, Polymorphism, Protein.

INTRODUCTION

Follicle stimulating hormone (FSH) is a gonadotropin hormone produced by the anterior pituitary gland that plays a role in the process of gametogenesis and steroidogenesis. The FSH can stimulate follicular development when binds to FSH receptor (FSHR) contained in the granulosa cell membrane in the ovary (Kang et al. 2010; Minegishi et al. 1997; Xu et al. 1995). The polymorphism of FSHR is one of the important factors that affect the role of FSH in the ovary. Therefore, the variant of FSHR must be considered to examine the fertility status, especially to identify a biomarker of animal reproduction. Follicle-stimulating hormone receptor is a G-protein coupled receptor that is expressed in granulosa cells where it mediates FSH signal transduction and follicle maturation (Simoni et al. 1997). The FSH bond with the extracellular domain of the FSH receptor stimulates a cascade of the intracellular process that leads to the occurrence of a specific response to FSH. The G protein activates adenylyl cyclase to stimulate cAMP synthesis that will activate kinase A-protein (Ulloa-Aguirre and Timossi, 1998). The interaction between FSH and FSHR can be analyzed by using bioinformatics or in silico technique (Ekins et al. 2007; Apwiler, 2001). In silico method has been widely used to study the relationship of the function of a protein with a molecular structure (Liu et al. 2008; Wielisch et al. 2006; Whitfield et al. 2006).

The polymorphism of FSHR gene exists on bovine (Cory et al. 2013; Gaviria et al. 2016), buffalo (Sosa et al. 2015; Othman and Abdel-Samad, 2013), human (Singhasena et al. 2014; Balkan et al. 2010), geese (Kang et al. 2010). The FSHR gene polymorphism mostly influenced reproduction performance, even though some of the references suggested that the variation not correlates with fertility rate. The variation in nucleotide sequences may lead to functional changes in protein (Yang et al. 2012). The gene polymorphisms linked to reproductive characteristics in beef cattle (Marson et al. 2008). FSH receptor variants appear to respond differently to FSH stimulation in vivo, they might play some role in determining ovarian response to pharmacological stimulation with FSH (Simoni et al. 2002). This study aims to elucidate the effect of FSHR polymorphism on its structure and function on Bos taurus through computational technology, in silico. The study was conducted based on the available data of FSHR sequences from Genbank. Moreover, the effects of polymorphisms on FSHR structure was examined using homology modeling method (swiss-server-model-based) and docking analysis. The study is a warrant for further investigation to explore biomarker of cattle reproduction status based on FSHR gene.

MATERIALS AND METHODS

Analysis of FSHR protein polymorphism: Amino acid sequence of the FSHR protein was downloaded from
FSHR and FSH protein structure modeling: The structure of follicle-stimulating hormone receptor (FSHR) and follicle stimulating hormone (FSH) were modeled by homology modeling method through Swiss server model. The models were constructed based on human FSHR and FSH protein from Protein Data Bank (PDB) (http://www.pdb.org/; ID: 1xwd). Then FSHR and FS models were validated by Ramachandran plot using Discovery Studio software (Eramian et al. 2006; Shen and Sali, 2006).

Interaction and binding affinity between FSH and FSHR: The binding affinity between FSHR and FSH (docking) was analyzed by using HEX server docking (Macindoe et al. 2010). The docking is set by a shape + electrostatic parameter and searches order 25. The binding domain and type of bond were analyzed by using dim plot (LigPlus) and visualized by using Discovery Studio software (Laskowski and Swindells, 2011).

Analysis of hydrophobicity and stability of FSHR variants: The structure of FSHR variants was constructed using FOLD-X software on YASARA by mutated the amino acid based on wild-type structure. The energy change and stability were calculated using FOLD-X on YASARA due to the point mutation (Schymkowitz et al. 2005). Then the hydrophobicity of the FSHR due to point mutation was analyzed by using Discovery Studio software. The stability of the structure and hydrophobicity is critical for a protein to bind strongly and stable with its ligand.

RESULTS AND DISCUSSION

All of FSHR sequences of Bos taurus were curated from gene and found only three variant of FSHR in the domain of FSH binding (amino acid residues 18 to 259). The variants located at amino acid residues 66 (F66L) and 113 (A113P) (Figure 1). The variant occurred at FSH binding domain; it can lead to changes in the binding pattern of FSHR into FSH (ligand). Moreover, the polymorphism may change the structure and properties of FSHR that affect the reproductive profile Bos taurus. Therefore, the structure FSHR protein variants were modeled to determine the effect of the polymorphism on its structure. The structure of three variants was modeled using Swiss-model and validated using Ramachandran plot with accuracy over 90% (Figure 2). The structure of the tree variant is similar or does not have any change in the backbone.

Further, we analyzed the binding pattern of the FSHR with FSH using docking method by Hex server software. Based on the docking results, it showed that variation of FSHR caused different binding patterns and binding affinity with FSH (Figure 3). The binding pattern of FSHR F66L variant into FSH has similar with wildtype but has the higher binding affinity. Conversely, A113P variant has different binding pattern and affinity compared to wild-type. This result suggested that the FSHR polymorphism has an impact on the pattern of binding and affinity to its ligand, FSH. This polymorphism also affects the amino acid differences that interact to form hydrogen bonds (Figure 3). The differences assumed will affect cascade signaling that controlled by FSH-FSHR interaction. The polymorphism allegedly has a role in cell communication mediated by FSH, such as fertility rates, and growth in Bos taurus. Based on the results indicated that the FSHR gene variation is very promising to be used as biomarkers of fertility in cattle.

Further, a deeper analysis of the impact of amino acid substitution of SFHR using FOLD-X simulation showed that the substitution amino acid residues 66 and 113 amino acid changed energy stability and hydrophobicity. Substitution amino acid from A into P (residue no 113) increases the stability of the protein (ddG -0808 kcal/mol). While substitution of F into L at amino acid residue 66 reduced stability of the protein (ddG 2868 kcal/mol). Moreover, the amino acid substitution also altered the hydrophobicity of surrounding of point mutation residue (Figure 4). The changes in the 113 amino acid from A into P will degrade hydrophobicity while the changes in the 66 amino acid from F into L will raise hydrophobicity of FSHR protein surface. The changes on the both properties might alter the binding pattern and binding affinity of FSHR into FSH. Moreover, the alteration of energy stability and hydrophobicity will lead to the stability of the structure of proteins and amino acids that interact with FSH.

The FSH receptor has a very significant role in communicating with FSH that regulates the reproductive system in a living creature, including cattle. The
Fig 2: The 3D structure of three FSHR variants (upper panel) and Ramachandran plots analysis showed high accuracy (over 90%) (bottom panel). The backbone of the tree variants is similar and almost does not any change. A: wild type, B: F66L variant and C: A113P variant.

Fig 3: The binding pattern of FSH (red) and FSHR (Blue) (left panel). The Hydrogen bond among amino acid of FSH and FSHR (right panel) and the detail amino acid that has hydrogen bound were represented in the table (bottom). A1= wild type, A2= A113P Variant, A3= F66L variant. B1= hydrogen bound on wild type, B2= hydrogen bound on wild type, B3= hydrogen bound on A113P variant, B3= hydrogen bound on F66L variant.

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Hydrogen bond</th>
<th>Bond Energy (Kcal/mol)</th>
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<tbody>
<tr>
<td>FSH: FSHR wildtype</td>
<td>D108: S76, V76: E30, L91: K96; E33: L144; E35: Q145; E38: N121</td>
<td>-2879.9</td>
</tr>
<tr>
<td>FSH: FSHR A113P</td>
<td>D108: S76, V76: E30, L91: K96; E33: L144; E35: Q145; E38: N121</td>
<td>-2888.2</td>
</tr>
<tr>
<td>FSH: FSHR F66L</td>
<td>Y121: K146, L1: K56, V76: E30; S107: E101; S109: R101; D111: R101; D112: Y124; E33: Q145</td>
<td>-2675.2</td>
</tr>
</tbody>
</table>

Physico Chemistry Parameter | Wild type | A113P | F66L |
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Total Stability (Kcal/mol)</td>
<td>171.88</td>
<td>170.17</td>
<td>173.29</td>
</tr>
<tr>
<td>DDG (Kcal/mol)</td>
<td>-</td>
<td>-0.899</td>
<td>2.859</td>
</tr>
<tr>
<td>The 66th Amino acid hydrophobicity</td>
<td>2.8</td>
<td>-</td>
<td>3.8</td>
</tr>
<tr>
<td>The 113th amino acid hydrophobicity</td>
<td>1.8</td>
<td>-3.5</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig 4: FSHR structure alignment between wildtype and A113P Variant (A); wild-type and F66L (B). The variants caused different side group formation (blue) compare to wild type (red). The variant also changed stability energy and hydrophobicity (table), which affected to binding affinity and binding pattern into FSH (ligand).

communication of FSH with the FSH receptor depends on the structure of these two molecules. The binding of FSH to the receptor, then the FSH receptor will undergo a conformational change that activates a cascade of various cell signaling including folliculogenesis. The FSH receptor protein structure must be compatible with its ligand (FSH). There of the FSH receptor gene variation might change shape and properties of the protein that affect strength and stability bond with the ligand, FSH. The changes in force and stability of binding between FSH and FSHR will affect cell communication that leads to disruption of communication among cells, such as disorders of the reproductive system. The result suggested that variation of FSHR has implication on cellular communication mediated by FSH that very promising for a biomarker of reproduction status of cattle (Bos Taurus).

CONCLUSION

Based on the results, FSHR of Bos taurus has three polymorphisms that located at amino acid residues 18th to 259th. The polymorphisms may alter its ability to bind with FSH. Molecular docking analysis indicated that all variant of FSHR potentially changes the pattern and affinity binding into FSH that may have an impact on reproduction status of Bos taurus. The study is a warrant for further investigation to explore biomarker of cattle reproduction status based on FSHR gene.
REFERENCES


