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In Silico Analysis of the Specificity of E-Prostanoid Receptors from Cayman® 101740, 101760, 101775 for Murine-Derived Kidney Tissues

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COLUMN ARTICLE

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Abbreviations: EP: Eprostanoid Receptor; PGE2: Prostaglandin E2

INTRODUCTION

The kidney plays a central role in regulating blood pressure, through a system called renin angiotensin hormonal axis (RAS) [1,2]. RAS components include renin (secreted by the juxtaglomerular cells against various stimuli), angiotensinogen, angiotensin I and angiotensin II, the latter is a potent vasoconstrictor and stimulates sodium reabsorption in the kidney [2-4], but this system that increases blood pressure is regulated in the kidney by prostaglandins, particularly PGE2, which intervenes on the vasoconstrictor effects and anti-natriuretic an activated RAS [5-7]. The PGE2 receptors act via 4 G protein-coupled receptors called E-prostanoids EP1, EP2, EP3 and EP4, being of great relevance physiological effects generated by EP1, EP3 and EP4 in the kidney [7]. For this reason, it is important to identify the EP1, EP3 and EP4 receptors and antibodies are used for this. Within the use of antibodies, it is very important to the specificity of these by the target protein and in this work, will investigate the specificity of antibodies Cayman Chemicals 101740, 101760 and 101775 for EP1, EP3 and EP4 receptors designed for recognition receptor E-Prostanoids human versus same receptors, but in mouse.

MATERIALS AND METHODS

To achieve our goal, amino acid sequences obtained from protein data base Uniprot (http://www.uniprot.org/) from different species were used, these are: *Homo sapiens, Mus musculus, Rattus norvegicus, Canis lupus familiaris, Bos taurus, Oryctolagus cuniculus, Sus scrofa, Pan troglodytes* and *Oryctolagus cuniculus;* and these were used to design a logo. The sequences corresponding to *Homo sapiens* and *Mus musculus* each receiver for the design of three-dimensional structures using the web server phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index).

RESULTS AND DISCUSSION

The results are shown in Figure 1. The corresponding developed logos to Figure 1B) shows that in terms amino-acidic conservation is concerned, the EP3 receptor is one that shows greater variability of amino acids, while one that has greater conservation is the EP1 receptor, suggesting at first instance, that a more specific recognition would be submitted by the antibody that recognizes EP1. Figure 1A) corresponds to a superposition of three-dimensional structures belonging to EP1, EP3 and EP4 receptors, where the sites corresponding to the nuclear membrane and

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cytoplasm are denoted. The images 1C), 1D) and 1E) correspond to a comparison between the receptors EP1, EP3 and EP4 (cyan) present in mice with respect to the same receptors present in human (blue), through an overlap of structures. In these images, we proceeded to identify the site that is recognized by the corresponding antibody and can identify that this differs according to the species (magenta, recognition site in mouse and red recognition site on human). Furthermore, we note that the location of the recognition site varies for each receiver, since EP1 and EP4 for receptors are inside the cell, whereas for EP3 is found in the extracellular region. These differences present in the recognition site could hinder the action of the antibody used. Furthermore, it has been reported by open literature that such receivers suffer from various glycosylations, which also hamper the interaction between the antibody and the target protein [8-11]. It has also been reported the expression of various isoforms for the EP3 receptor [12], which hinders the development of specific antibodies and recognition of these membrane proteins.

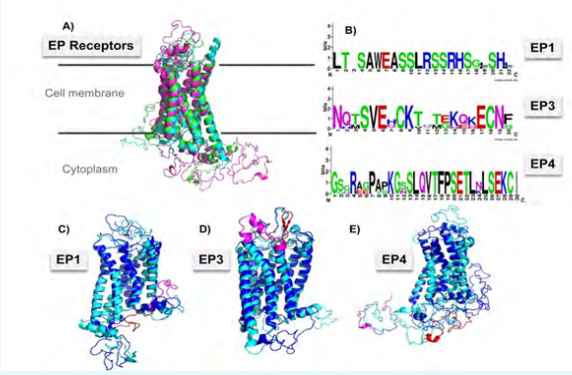

Figure 1: *In silico* analysis of primary antibodies Cayman 101740, 101750 and 101775, for E-prostanoid receptors EP1, EP3 and EP4. Figure A corresponds to the (green) three-dimensional figures of EP1 (green), EP3 (cyan), EP4 (magenta) created from the web server phyre2, these are positioned from the amino terminus (extracellular domain) to the carboxyl terminus (domain intracellular). B) corresponds to the design of logos. Figures C), D) and E) correspond to the superposition of the three-dimensional structures of E-prostanoids receptors corresponding to the species *Mus musculus* (cyan) and *Homo sapiens* (blue).

CONCLUSION

These results could suggest that there is no complete specificity for the developed antibodies to recognize mouse EP1, EP3 and EP4 receptors, so that additional analysis is necessary to achieve a correct interpretation of the results obtained in the laboratory.

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CONFLICT OF INTEREST

No conflict of interest.

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