Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *https://doi.org/10.17952/36EPS.2022.079*

Study on the Possibility of Cross-Interactions of Selected Growth Factors with Specific and Non-Specific Antibodies

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Introduction

Proteins are the basic building blocks of living organisms. They also play a key role in a variety of biological processes, such as catalyzing chemical reactions, transporting various compounds, immunological reactions, and signaling between cells [1]. All biological processes are regulated by protein complexes, the activity of which is controlled by protein-protein interactions (PPI) [2,3]. A specific type of PPI is the interaction between antibodies and protein antigens. Essentially, each individual antibody is able to bind specifically to one unique epitope due to a unique antigen-binding site located at the end of the variable region on the antibody. However, an epitope (linear or conformational) may be present in more than one protein antigen. Thus, one antibody can potentially recognize two or more proteins if the proteins are highly homologous and contain the same epitope. This results in the possibility of cross-interaction with other proteins [4-6]. It is commonly believed that the molecular mimicry of epitopes is the cause of the development of many autoimmune and neoplastic diseases [7-11].

Growth factors are specialized proteins (polypeptides) involved in intercellular communication. They activate the body's repair mechanisms. Among the growth factors, over 30 compounds can be distinguished. The most commonly used ones in widely understood medicine are Epidermal Growth Factor (EGF), Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF), Fibroblast Growth Factor (FGF), and Transforming Growth Factor (TGF). EGF is a common mitogenic factor that stimulates the proliferation and differentiation of various cell types, especially fibroblasts and epithelial cells, and accelerates wound healing. EGF activates the EGF receptor (EGFR/ErbB), which in turn initiates intracellular signaling. EGF supports the differentiation, maturation, and survival of different neurons as well and has a neuromodulatory effect on different types of neurons in the CNS [12,13]. Transforming growth factor alpha (TGF-α) belongs to the epidermal growth factor family. TGF stimulates fibroblasts to produce collagen and stimulates DNA synthesis, proliferation, and differentiation of various types of cells. The expression of TGF-α is highly regulated in response to exogenous cellular signals, including cytokines and other growth factors. This growth factor has been found to be essential for the proper development of many tissues and organs. TGF-α also plays a role in many neoplastic diseases [14]. VEGF stimulates the processes of angiogenesis, i.e. the formation of blood vessels, and activates microcirculation. VEGF is considered a major regulator of angiogenesis in disease states such as cancer, diabetes, and macular degeneration [15,16]. By acting on the appropriate receptors or their isoforms, growth factors trigger cascades of various processes in the body [17-19] which are either harmful or constitute targets in the design of new drugs [20-23]. On the other hand, crosstalk between growth factors and other proteins is widely known, resulting in the formation of new networks of protein-protein interactions and ultimately obtaining different biological responses [24-28].

As a result of our research on the selection of protein fragments involved in the regeneration process of damaged tissues, we have shown [29] that for selection it is possible to use polyclonal antibodies specific to a protein, because finally the set of fragments forming the outer sphere of the protein is obtained. This is due to the fact that the amino acid sequence is important in the recognition of an antigen by antibodies, but a very important factor determining the interaction is the appropriate display of protein fragments, which allows the formation of an antigen-antibody complex. Additionally, polyclonal antibodies are known to recognize many antigens of the one antigen.

The aim of this study was to test the ability to interact with overlapping decapeptide libraries covering entire growth factors such as epidermal growth factor, transforming growth factor alpha, and vascular endothelial growth factor A with specific polyclonal antibodies and non-specific antibodies. Conducting these studies should provide answers to questions about possible cross-interactions between anti-growth factor antibodies and native growth factors. Finding growth factor fragments

incorrectly recognized by anti-growth factor antibodies should, on the one hand, broaden the knowledge of their possible application, as well as provide an answer to the possibility of non-specific interaction of growth factor fragments with various receptors. Experimental studies based on dot-blot tests of libraries of overlapping decapeptides constituting maps of proteins from the growth factor family with antibodies were extended to modeling the interactions of growth factor fragments with selected receptors. It was assumed that both "correct pairs" (ligand-receptor) and "incorrect pairs" (non-specific ligand-receptor) would be used in further research.

Results and Discussion

In the first stage of the research, the syntheses of libraries of fragments of the selected growth factors immobilized on the cellulose surface were carried out. The libraries of fragments of TGF-α, EGF, and VEGF A were obtained. The first stage of the synthetic work involved the incorporation of the isocyanurate derivative on the surface of the cellulose matrix [30,31] (Figure 1). As our research has shown, the isocyanurate derivatives can be used in the SPOT peptide synthesis (modified cellulose as a solid phase) according to the Fmoc/tBu strategy. By using piperidine, it was possible to remove the Fmoc group, which makes it suitable to continue peptide synthesis according to the classical solid phase synthesis procedure. Synthetic protocols [32] were adapted to the synthesis of libraries of decapeptide fragments derived from TGF-α, EGF, and VEGF A. Libraries of decapeptides being overlapping fragments of selected growth factors (reading frame shifted by 1 amino acid residue) was synthesized. Synthesized libraries consisted of 41 fragments for TGF-α, 44 fragments for EGF, and 233 fragments for VEGF A. These libraries were used in subsequent stages to study the ability of interaction with polyclonal antibodies (both specific and non-specific). To visualize peptide-antibody complexes secondary antibodies labeled with horseradish peroxidase (HRP) were used. In order to test the ability of synthesized libraries to interact with specific or non-specific antibodies against growth factors, polyclonal anti-EGF, anti- TGF-α, and anti-VEGF A were used. All performed reactions either with specific or non-specific antibodies showed that there are recognizable decapeptides in each setup (Figure 2).

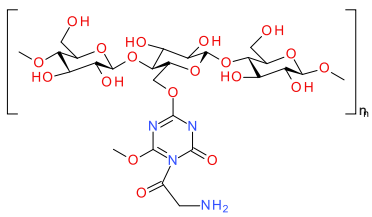

Fig. 1. Isocyanurate derivative immobilized on cellulose prior to peptide synthesis.

EGF and TGF-α belong to growth factors that interact with the same receptor (EGFR), which of course results in the activation of the same processes in the cell [33,34]. Despite the fact that EGF and TGF-α bind to the same receptor, reactions with antibodies specific to them allowed for the selection of structurally diverse fragments. For both EGF and TGF-α 4 fragments were selected. As a result of interactions of the library of EGF-derived decapeptides with anti-TGF-α antibodies 4 fragments were selected, and for TGF-α-derived decapeptides with anti-EGF antibodies it was 1 fragment was found (Figure 3). These fragments partially overlap. In contrast, the fragment 29-38 EGF recognized by anti-TGF-α antibodies is not identified by anti-EGF antibodies, although it partially overlaps with the adjacent active fragments. It can be found that overlapping fragments contain residues conserved for EGF-family proteins. Apart from these residues, there are also others that are similar or the same for both sequences.

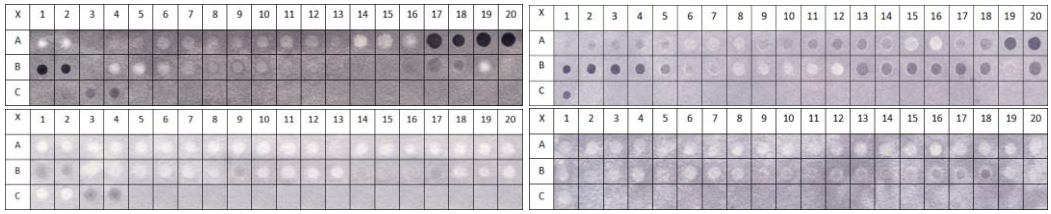

Fig. 2. Exemplary scans of cellulose matrices with peptide libraries of EGF (left) and TGF-α (right) after reactions with polyclonal antibodies: specific (top) and non-specific (bottom).

Analyzing this fact, it can be concluded that the spatial structure of EGF and TGF-α molecules allows for the exposition of similar fragments, which is visible in the above list of fragments selected in reactions with specific antibodies. Binding to the receptor as well as immunization in the process of obtaining antibodies is conditioned by the spatial structure and the appropriate amino acid sequence. It has been found that, despite the presence of a few constant amino acid residues in both sequences, the differences that exist may be of crucial importance in antibody formation. Hence the fact that despite binding to the same receptor, the results of the reaction with EGF and TGF-α antibodies are not identical. For VEGF A interaction with specific polyclonal antibodies resulted in six recognizable fragments. VEGF A belongs to another family of growth factors, but reaction with non-specific antibodies showed, that its fragments may be misrecognized by anti-EGF and anti-TGF-α antibodies. The conducted studies with non-specific anti-EGF and anti-TGF-α antibodies clearly showed that as a result of an incorrect recognition, a significantly greater number of VEGF fragments were selected, than with specific anti-VEGF antibodies. It is worth emphasizing that all selected fragments using specific anti-VEGF antibodies were also found in the dot-blot test with non-specific antibodies. It is also interesting that the VEGF fragments recognized by anti-EGF and anti-TGF-α antibodies were highly compatible. Even for structurally different fragments of the VEGF165 isoform (no reaction with specific anti-VEGF antibodies, one fragment was selected in the test with anti-EGF and anti-TGF-α antibodies. What is surprising, is the almost complete structural homology of the fragments found (you only move one amino acid). Some similarities can be found by analyzing the sequences of the identified fragments. The EGF fragment 33-47 and the TGF-α fragment 29-47 recognized by the anti-EGF antibodies are largely identical. This sequence also includes the aminoacids constant for proteins from the EGF family. Similar sequence is also found in VEGF A 166-175 fragment.

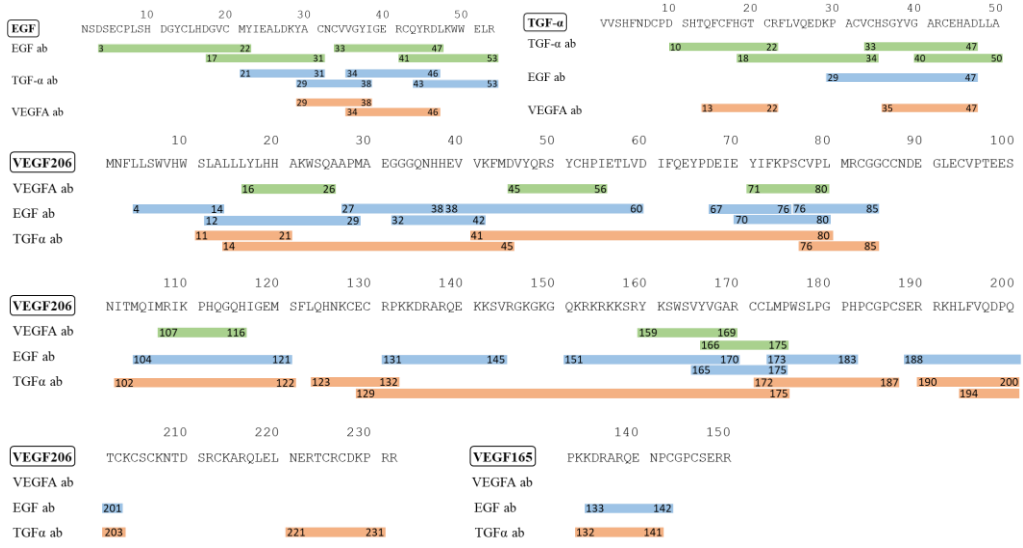

Fig. 3. Graphic scheme of interactions between protein fragments and specific/non-specific polyclonal antibodies.

The results of studies on the cross-interaction of anti-EGF and anti-TGF-α antibodies with VEGF indicate a very high probability of incorrect recognition of growth factors by non-specific antibodies, produced against structurally and functionally different growth factors. Dividing proteins into short peptides show that even between those from different families structural similarities/homology may be found. This fact may indicate that these structures can possibly activate improper proteins during PPI's. Also, studies on mistaken interactions open a new way to design new, unexpected peptides able to interact with selected proteins/receptors.

Acknowledgments

The research was funded by the National Science Centre, Poland, grant number UMO-2018/31/B/ST8/02760.

References

1. Lu, H., et al. *Signal Transduct. Target Ther*. **5**, 213 (2020), https://doi:10.1038/s41392-020-00315-3
2. Stelzl, U., et al. *Cell* **122**, 957-968 (2005), https://doi:10.1016/j.cell.2005.08.029
3. Rual, J.F., et al. *Nature* **437**, 1173-1178 (2005), https://doi.org/10.1038/nature04209
4. Augustyniak D., et al. *Curr Med. Chem*. **24**, 4002-4037 (2017), https://doi:10.2174/0929867324666170508110222
5. Jain, D., et al. *Biochem. J*. **476**, 433-447 (2019), https://doi:10.1042/BCJ20180670
6. Leach, M.W., et al. *Toxicol. Pathol*. **38**, 1138-1166 (2010), https://doi:10.1177/0192623310382559
7. Wildner, G., et al. *Front. Immunol*. **11**, 580636 (2020), https://doi:10.3389/fimmu.2020.580636
8. Rees, A.R., *MAbs* **12**, 1729683 (2020), https://doi:10.1080/19420862.2020.1729683
9. Peng, H.-P., et al. *PNAS* **111**, E2656-E2665 (2014), https://doi.org/10.1073/pnas.1401131111
10. Chiaro, J., et al. *Cancer Immunol. Res.* **9**, 981-93 (2021), https://doi.org/10.1158/2326-6066.CIR-20-0814
11. Eisen, H.N. *Annu. Rev. Immunol*. **19**, 1-21 (2001), https://doi.org/10.1146/annurev.immunol.19.1.1
12. Schneider, M.R., et al. *J. Cell. Physiol*. **218**, 460-466 (2009), https://doi:10.1002/jcp.21635
13. Wong, R.W.C., et al. *Cytokine Growth Factor Rev*. **15**, 147-156 (2004), https://doi:10.1016/j.cytogfr.2004.01.004
14. Booth, B.W., et al. *Growth Factors* **25**, 227-235 (2007), https://doi.org/10.1080/08977190701750698
15. Nieves, B.J. *BioFactors* **35**, 332-337 (2009), https://doi.org/10.1002/biof.46
16. Corvol P. *Bull. Acad. Natl. Med*. **192**, 289-300 (2008), PMID: 18819684
17. Peach, C.J., et al. *Int. J. Mol. Sci*. **19**, 1264 (2018), https://doi:10.3390/ijms19041264
18. Chen, J., et al. *Physiol. Rev*. **96**, 1025-1069 (2016), https://doi:10.1152/physrev.00030.2015
19. Singh, B., et al. *Semin. Cell Dev. Biol*. **28**, 12-21 (2014), https://doi:10.1016/j.semcdb.2014.03.003
20. Mitchell, R.A., et al. *Exp. Cell Res*. **371**, 1-19 (2018), https://doi:10.1016/j.yexcr.2018.08.009
21. Shaik, F., et al. *Biomolecules* **10**, 1673 (2020), https://doi:10.3390/biom10121673
22. Martin-Fernandez, M.L., et al. *Cells* **8**, 316 (2019), https://doi:10.3390/cells8040316
23. Ferrara, N. *Trans Vis. Sci. Tech.* **5**, 10 (2016), https://doi:10.1167/tvst.5.2.10
24. Latko, M., et al. *Cells* **8**, 455 (2019), https://doi:10.3390/cells8050455
25. Luo, K. *Cold Spring Harb Perspect. Biol*. **9**, a022137 (2017), https://doi:10.1101/cshperspect.a022137
26. Tsuda, T. *Int. J. Mol. Sci*. **19**, 2787 (2018), https://doi:10.3390/ijms19092787
27. Munger, J.S., et al. *Cold Spring Harb Perspect. Biol*. **3**, a005017 (2011), https://doi:10.1101/cshperspect.a005017
28. Bjorkelund, H., et al. *PLoS ONE* **6**, e16536 (2011), https://doi.org/10.1371/journal.pone.0016536
29. Kolasa, M., et al. *Int. J. Mol. Sci*. **22**, 13584 (2021), https://doi.org/10.3390/ijms222413584
30. Kaminski, Z.J., et al. *Chem. Biodiv*. **14**, e1700444 (2017), https://doi:10.1002/cbdv.201700444
31. Bak, A., et al. *Comb. Chem. High Throughput Screen*. **17**, 141-156 (2014), https://doi:10.2174/1386207313169990054
32. Fraczyk, J., et al. *J. Pep. Sci*. **24**, e3063 (2018), https://doi:10.1002/psc.3063
33. Winkler, M.E., et al. *Biochemistry* **28**, 6373-6378 (1989), https://doi:10.1021/bi00441a033
34. Ebner, R., et al. *Cell R*egul. **2**, 599-612 (1991), https://doi:10.1091/mbc.2.8.599