

Development of Improved Methods for Protein Separation and Identification

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Abstract — For identification of proteins, able to connect specifically or non-specifically with peptide SCGN, protein material from RIN-5F rat insulinoma malignant cells and *E. coli* bacteria strains, both with inserted by transfection with recombinant vectors rat gene *SCGN*, was isolated. Subsequently, SCGN peptide was isolated, precipitated and incubated with lysates from rat pancreas and brain, known as anatomic organs with highest expression of *SCGN* gene. For determination of proteins with highest affinity to this target peptide, from both organs, it was separately mixed with both lysates, and the mixtures were subjected on separation on CNBr-Sepharose and GST-Agarose columns, respectively. GST-Agarose technique indicated some advantages in comparison with CNBr-Sepharose, connected mainly with higher yields of different protein types because of the escaped protein degradation influence of CNBr. The results supported the suggested in our previous studies abilities of protein SCGN to connect with cytoskeleton elements, in confirmation with some literature findings. On this base, a mechanism for indirect influence of this peptide on the control of cell growth and proliferation, has been proposed. Future studies are necessary in this direction.

Keywords — Protein Separation, Protein Identification, Cytoskeleton Proteins, Protein-Protein Interactions.

I. INTRODUCTION

Evidence for involvement of peptide Secretagoin (SCGN) in the control of cell growth, proliferation and transformation has been proposed [1]-[5]. This molecule has recently been cloned and characterized as beta-cell-expressed EF-hand Ca^{2+} -binding protein [4]-[7]. Functional analysis of transfected cell lines, expressing recombinant SCGN, has revealed an influence on calcium flux and cell proliferation. Such regulation could be achieved via direct protein-protein interactions [1]. As an example, in RIN-5F cells (rat Langerhans islet malignant cell line), an anti-proliferative effect has been identified, which is probably due to Secretagoin-triggered down-regulation of substance P transcription [6]. At the same time, a calcium-dependent SCGN-TAU interaction, as well as co-induction of TAU, has been found in the islets of Langerhans and beta-cell-derived cell lines with high expression of the neuroendocrine-specific protein SCGN [5]. In that direction, investigations about eventual direct and/or indirect influence of protein SCGN on nuclear proteins functions by cascade regulatory mechanisms have been proposed [8].

In this attitude, for investigation of specific proteins from rat pancreas and brain with high affinity with recombinant rat peptide SCGN, used in its role of tumor suppressor, two different techniques of protein separation were tested and compared.

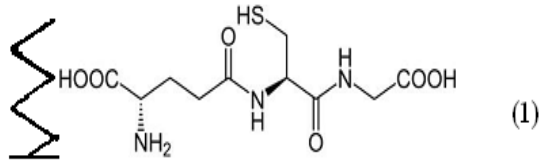
II. MATERIALS AND METHODS

Recombinant target rat peptide SCGN, isolated from transfected with recombinant DNA-vectors malignant RIN-5F rat insulinoma cells and *E. coli* bacteria strains, respectively, was incubated in protein lysates from rat pancreatic and brain, as anatomic organs, known as the most actively expression of *SCGN* gene in building cells. Because of the revealed very near protein profiles in both organs, different concentrations of the isolated from them proteins were also used in the current study – protein lysate from rat pancreas was diluted 10 folds. Subsequently, bacteria and both organs were treated with lysis buffer and put on $4^{\circ}C$ for 2 hours. After addition of specific anti-rat SCGN antibody to the so obtained cell culture and bacteria cell protein lysates, and subsequent centrifugation, the pellet was resuspended in PBS. The so prepared protein suspensions were then mixed with the obtained protein lysates from rat brain and rat pancreas, respectively, and both mixtures were subjected on CNBr-Sepharose and GST-Agarose columns, respectively, for 2 hours with intensive shaking for connection of proteins with affinity to the recombinant rat peptide SCGN of both organs from the other proteins in them. After elution of protein materials from both columns with SDS and LDS (Lithium dodecylsulphate) buffers, the so prepared probes were put at $70^{\circ}C$ for 10 minutes, and subsequent addition of LDS-leading buffer and Reducing agent, they were separated by LDS-Polyacrylamide Gel Electrophoresis.

III. RESULTS AND DISCUSSION

Fig. 1 shows the distribution of proteins from rat brain and pancreas lysates on molecular weight, as well as the distribution of proteins both anatomic organs, to which recombinant rat SCGN protein, incubated lysates of these organs, is specifically or non-specifically connected, respectively. These data supported the usefulness of the used method for precise establishment of novel protein-protein molecular interactions, but also confirmed the results of our previous studies about the affinity of peptide to cytoskeleton components [8], which were additionally supported by the electrophoretic profiles of protein lysates different dilutions from two anatomic organs because of the established very near protein composition of them [9], [10]. These results supported our previous data about predominating cytoskeleton proteins with the highest affinity to peptide SCGN in both anatomic organs (Fig. 2 – b, c) [8], which were also in agreement with literature findings about its participation in cascade regulatory mechanisms on the processes of cell growth and proliferation, by protein-protein interactions [1], [2], [5]-[7], [11]-[13]. Additionally, the current study indicates

some advantages of the applied technique about protein separation by GST-Agarose (Figure 2), in comparison with CNBr-Sepharose, which was probably connected with the escaped protein degradation on the influence of CNBr, as well as, on the other hand, due to the characteristic in the chemistry structure of the reduced Glutathione (GSH) [14].



Sepharose Reduced Glutathione (GSH)

These data confirmed our previous data about the analogy of RIN-5F malignant rat insulinoma cells with HeLa malignant human cells, on the one hand, as well as of tumor-suppressor genes *SCGN* and *HACE1*, on the other. In this way, these data were also in agreement with many literature findings, indicating many similarities in molecular mass and absorption spectra of cytoskeleton elements, observed in the current study [2], [9], [15]-[18]. The results obtained also supported the usefulness of the used method for precise establishment of novel protein-protein molecular interactions and confirmed the results of our previous studies about the affinity of peptide to cytoskeleton components [15], which was in agreement with the literature data [9], [18]-[20]. On the other hand, these data indicated a lot of advantages of GST-Agarose technique for protein separation in comparison with CNBr-Sepharose technique, mainly connected with higher yields of different protein types because of the escaped protein degradation influence of CNBr, which is also in agreement with some literature findings [14].

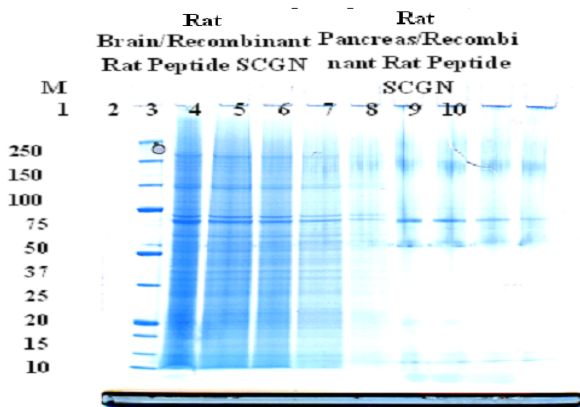


Fig.1. LDS-PAGE of proteins, isolated rat brain and pancreas with incubated recombinant rat protein SCGN in them: lanes 2-6: recombinant rat SCGN protein, incubated in protein lysate from rat brain; lanes 7-10: recombinant rat protein SCGN, incubated in protein lysate from rat pancreas.

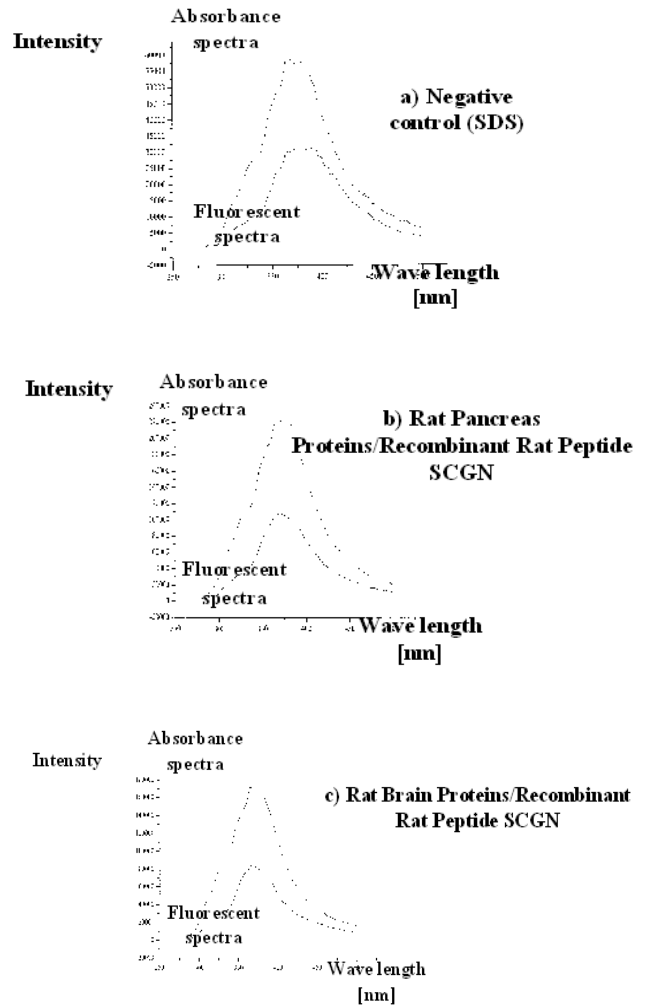


Fig.2. (A to C): Intensity of absorbance and fluorescent spectra in different wave length values: negative control of SDS in the absence of protein material (a); proteins from rat brain, able to connect with recombinant rat peptide SCGN (b); proteins from rat pancreas, able to connect with recombinant rat peptide SCGN (c).

IV. CONCLUSION

For establishment of specific proteins with high affinity to the tested target peptide SCGN, future investigations for comparison of with control protein profiles from brain and pancreas with these with SCGN peptide, connected with proteins from each one of both anatomic organs, are necessary. For this goal, GST-Agarose technique for protein separation should be followed by label-free LC-MS/MS assay with subsequent applications of computer programs and statistic analyses for protein identification and quantification.

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REFERENCES

- [1] M. C. Bauer, D. J. O'Connell, M. Maj, L. Wagner, D. J. Cahill, and S. Linse, "Identification of a high-affinity network of secretagogin-binding proteins involved in vesicle secretion." *Mol. BioSyst.*, vol. 7, pp. 2196-2204, 2011.
- [2] K. A. Butner, M. W. Kirschner, "Tau protein binds to microtubules through a flexible array of distributed weak seats." *J. Cell Biol.*, vol. 115, pp. 717-730, 1991.
- [3] W. Gartner, G. Vila, T. Daneva, A. Nabokikh, F. Koc-Saral, A. Ilhan, O. Majdic, A. Luger, and L. Wagner, "New functional aspects of the neuroendocrine marker secretagogin based on the characterization of its rat homolog." *Am. J. Physiol. Endocrinol. Metab.*, vol. 293, pp. E347-E354, 2007.
- [4] L. Wagner, O. Oliyarnyc, W. Gartner, P. Nowotny, M. Groeger, K. K. Waldhäusl, and M. S. Pasternack, "Cloning and expression of secretagogin, a novel neuroendocrine- and pancreatic islet of Langerhans-specific Ca^{2+} -binding protein." *J. Biol. Chem.*, vol. 275, pp. 24740-24751, 2000.
- [5] M. Maj, W. Gartner, A. Ilhan, D. Neziri, J. Attems, and L. Wagner, "Expression of TAU in insulin-secreting cells and its interaction with the calcium-binding protein secretagogin." *J. Endocrinol.*, vol. 205, pp. 25-36, 2010.
- [6] A. Rogstam, S. Linse, A. Lindqvist, P. James, L. Wagner, and T. Berggard, "Binding of calcium ions and SNAP-25 to the hexa EF-hand protein secretagogin." *Biochem. J.*, vol. 401, pp. 353-363, 2007.
- [7] D. R. Witcher, M. De Waard, H. Liu, M. Pragnell, and K. P. Campbell, "Association of native Ca^{2+} channel subunits with the γ 1 subunit interaction domain." *J. Biol. Chem.*, vol. 270, pp. 18088-18093, 1995.
- [8] I. Sainova, I. Vavrek, V. Pavlova, T. Daneva, Sh. Pashova, E. Nikolova, "Experimental *in vitro*-human and animal models for balanced activity of oncogenes and tumor-suppressor genes and their protein products." *Schol. J. Biotechnol.*, vol. 1, pp. 8-14, 2012.
- [9] H. Putaala, R. Slnnen, P. Klipeläinen, J. Wartlovaara, and K. Triggvason, "The murine nephrin gene is specifically expressed in kidney, brain and pancreas: inactivation of the gene leads to massive proteinuria and neonatal death." *Hum. Mol. Genet.*, vol. 10, pp. 1-8, 2001.
- [10] Y. Takeda, M. Hoshino, N. Yanaihara, C. Yanaihara, J. Isobe, N. Sugiura, K. Kashimoto, Y. Takano, and H. Kamyia, "Composition of CCK8 receptors in the pancreas and brain in rats using CCK8 analogues." *Jpn. J. Pharmacol.*, vol. 49, pp. 471-481, 1989.
- [11] E. Aamodt, J. G. Culotti, "Microtubules and microtubule-associated proteins from the nematode *Caenorhabditis elegans*: periodic cross-links connect microtubules *in vitro*." *J. Cell Biol.*, vol. 103, pp. 23-31, 1986.
- [12] B. Buchwitz, K. Ahmad, L. L. Moore, M. B. Roth, and S. Henikoff, "Cell division: a histone-H3- like protein in *C. elegans*." *Nature*, vol. 401, pp. 547-548, 1999.
- [13] J. Goncalves, M. Graos, and A. X. C. N. Velente, "Polar mapper: computational tool for integrated visualization of protein interaction networks and mRNA expression data." *Nature Proceedings*, vol. 6, pp. 881-896, 2009.
- [14] P. Cuattrecasas, "Protein purification by affinity chromatography: derivatizations of agarose and polyacrylamide beads." *J. Biol. Chem.*, vol. 245, pp. 3059-3065, 1970.
- [15] M. Fischer, I. Haase, E. Simmeth, and G. G. Müller-Taubenberger, "A brilliant monomeric red fluorescent protein to visualize cytoskeleton dynamics in *Dictyostelium*." *FEBS Letters*, vol. 577, pp. 227-232, 2004.
- [16] Z. N. Gheshlaghi, G. H. Riazi, S. Ahmadian, M. Ghafari, and R. Mahinpour, "Toxicity and interaction of titanium dioxide nanoparticles with microtubule protein." *Acta Biochim. Biophys. Sin.*, vol. 40, pp. 777-782, 2008.
- [17] A. Khalegian, G. H. Riazi, S. Ahmadian, M. Ghafari, M. Rezaie, A. Takahashi, Y. Nakaya, and H. Nazari, "Effect of ferric oxide nanoparticles on microtubules organization." *Afr. J. Biochem. Res.*, vol.4, pp. 99-104, 2010.
- [18] L. Li, D. Zhao, C. Zhang, Q. Zhang, and S. You, "Procarcotic expression and polyclonal antibody preparation of novel ZLG10 protein involved in infection of RSV on SPC-A1 cells." *Prot. Expr. Purif.*, vol. 41, pp. 170-176, 2005.
- [19] G. E. Lindreth, G. D. Rieser, "Nerve growth factor- and epidermal growth factor-stimulated phosphorylation of PC12 cytoskeletally associated protein *in situ*." *J. Cell Biol.*, vol. 100, pp. 677-683, 1985.
- [20] M. Rockstroch, S. A. Müller, C. Jende, A. Kerzhner, M. von Bergen, and J. M. Tomm, "Cell fraction – an important tool for compartment proteomics." *J. Int. Omics*, vol. 1, pp. 135-143, 2011.