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Mini-Review

Secretagogin: Multiple aspects of a novel neuroendocrine marker

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ABSTRACT

Calcium binding proteins are centrally involved in mediating intracellular calcium signals. Secretagogin (*SCGN*) is a recently characterized hexa-EF hand calcium binding protein which was cloned from a beta-cell cDNA library. Two full-length variants (Secretagogin-R22 and Secretagogin-Q22) and a truncated variant (Setagin), which exhibits no calcium binding capacity, have been identified so far. Full-length *SCGN* binds four calcium ions with a $[Ca2+]_{0.5}$ of approximately 25µM and exhibits structural changes following calcium binding. Therefore *SCGN* was confined to be rather a calcium sensor than a calcium buffer protein. *SCGN* is highly and specifically expressed in neuroendocrine cells, especially in pancreatic beta cells. Additional expression was found in distinct neurons of the central nervous system and in a subgroup of colorectal cancers. Functional in vitro analysis and its interaction with the SNARE complex member SNAP-25 implicate its involvement in the insulin secretion process. Basing on its characteristic expression pattern, *SCGN* was implemented as neuronendocrine marker protein. Moreover, *SCGN* is detectable in the serum of patients suffering from cerebral hypoxia. In this context, recently developed commercial kits for the serum detection of *SCGN* allow its clinical application. In conclusion, *SCGN* is a recently identified calcium binding protein with functional and clinical aspects in the field of neuroendocrinology.

KEYWORDS: Secretagogin, calcium-binding proteins, neuroendocrine marker

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INTRODUCTION

In excitable cells with secretion activity, such as endocrine cells and neurons, intracellular calcium fluctuations are in the centre of the second messenger signalling pathways, finally resulting in the assembly of the secretion machinery, granule trafficking and exocytosis [1]. Additionally, intracellular calcium signals are involved in transcription regulation, growth control and apoptotic processes [2-4]. In the transmission of these intracellular calcium signals, calcium binding proteins are of central importance [5].

Characterization of Secretagogin

Immunoscreening of a human beta-cell cDNA library using a recently generated, beta-cell specific monoclonal antibody (mAb D24), resulted in the cloning of a novel member of the EF-hand calcium binding protein family, which was termed Secretagogin [6, 7]. EF-hand calcium binding proteins share a unique tandem repeat of a calcium binding loop flanked by two alpha helices. Due to its tertiary structure, this calcium binding site is called the “EF-hand” [8]. Bioinformatic analysis, comparative sequence analysis and calcium overlay experiments

revealed a considerable calcium binding capacity of full-length secretagogin (*SCGN*), which is based on 6 EF-hand calcium binding sites [6, 9]. Notably, the individual EF-hand calcium binding sites are characterized by specific and varying calcium binding capacities, with EF-hand motifs 1 and 2 completely lacking calcium binding properties [10, 11]. Due to its rather low calcium affinity with a $[Ca2+]_{0.5}$ of approximately 25µM and a significant change of its tertiary structure upon calcium binding with an increased exposure of hydrophobic surface, *SCGN* was emphasized to be rather a calcium sensor than a calcium buffer [10, 11]. Using the newly generated antibody mAb D24 in immunoblot and immunostaining experiments as well as the newly identified coding sequence of *SCGN* in mRNA based tissue expression analysis, we demonstrated a highly characteristic neuroendocrine tissue expression pattern of *SCGN*. Highest expression levels were found in the pancreatic islets of Langerhans, where the expression intensity culminated within the beta-cells. Additionally, *SCGN* expression was present in the adrenal gland, the C-cells of the thyroid and in neuroendocrine cells of the gastrointestinal tract [6]. Moreover, *SCGN* was expressed in the central nervous system, where it was found to a high extent in basket and stellate cells of the cerebellar cortex, in secretory neurons of the anterior part of the pituitary gland and in singular neurons of the frontal and parietal neocortex as well as in the hypothalamus and hippocampus [12]. Within the hippocampus, *SCGN* was restricted to pyramidal neurons and showed a hierarchical distribution depending on the individual sectors [13]. The data on *SCGN* expression in the central nervous system were extended most recently and demonstrated a highly specific neuronal distribution pattern allowing the identification of neuronal subtypes and hierachical organizing principles within the human brain. Interestingly, comparative expression analysis revealed a considerable inter-species variability of the *SCGN* expression pattern within the brain [14]. Moreover, *SCGN* was found in cone bipolar cells of the mammalian retina [15].

Bioinformatic sequence analysis predicted a molecular weight of 32kDa for *SCGN*. This was confirmed by immunoblotting, using the mAb D24 as well as a newly generated polyclonal rabbit anti-*SCGN* antibody [6]. Human *SCGN* is coded by a gene (gene bank accession number Y16752) located at chromosome 6. The coding sequence is characterized by 11 exons consisting of 828 base pairs. Additionally, we identified two *SCGN* protein variants [9]. On the one hand, out-splicing of exons 2 and 7 leads to a frame shift and induction of a pre-terminal stop codon after codon 49 (Setagin; gene bank accession number AF420280). On the other hand, a base-pair exchange within codon 22 (G/A), which is most probably due to RNA-editing, results in an aminoacid exchange (Q/R) with expression of *SCGN*-R22. To improve the opportunities for functional analysis of *SCGN* in rodent systems, we characterized its rat homolog [16]. Sequence analysis, immunostaining and immunoblotting using a rat secretagogin specific antibody demonstrated a high degree of sequence homology of human *SCGN* and its rat homolog (*Scgn*; gene bank accession number: AY513659) as well as comparable tissue expression patterns. The highest sequence variability between human and rat secretagogin was found at the N-terminus. This resulted in loss of the two N-terminal EF-hand motifs. Notably, despite their EF-hand structure, these protein parts exhibit only negligible calcium binding capacity [11], indicating the conserved calcium binding potential of *Scgn*. In contrast to the human isoform, in rat derived tissues no additional protein variants were yet identified. Immunofluorescence staining and subsequent confocal microscopy as well as cell fractionation experiments using sucrose gradients revealed a predominantly cytoplasmic intracellular localization of *Scgn*, although a minority of the intracellular *Scgn* content was also found at intranuclear sites and within granular cell compartments. Notably, in the insulin expressing rat insulinoma cell line Rin-5F and in tissue sections of rat pancreatic islets, co-localization of *Scgn* and insulin was demonstrated.

Functional aspects of Secretagogin in endocrine cells

The highly specific expression of *SCGN* in excitable, secretory active cells, its calcium binding properties and its calcium sensor characteristics implicate involvement in the modulation of intracellular calcium dependent

processes. Aiming at the elucidation of functional aspects of *SCGN*, cell culture based experiments using the wild-type rat insulinoma cell line Rin-5F as well as *SCGN* over-expressing Rin-5F cells, which were generated by transfection, were performed [6, 16]. Interestingly, a KCl-induced calcium flux was markedly and significantly increased in *SCGN* over-expressing Rin-5F cells when compared with sham transfectants. This highlights the influence of *SCGN* on intracellular calcium signalling. The increased KCl-inducible calcium flux observed in *SCGN* overexpressing Rin-5F clones was paralleled by an exaggerated insulin transcription and insulin secretion rate. In this respect, the specific interaction of *SCGN* with SNAP-25, which is influenced by calcium, has to be stressed [11]. SNAP-25 is a member of the SNARE complex and is thus centrally involved in calcium-dependent secretion processes. Underlining the multimodality of calcium signalling and thus of calcium binding proteins, *SCGN* overexpressing Rin-5F cells were characterized by a decreased growth rate. This might be due to the additionally observed *SCGN*-triggered down regulation of substance P transcription [6]. Using a *Scgn* specific sandwich-capture ELISA, we demonstrated increased *Scgn* release from viable Rin-5F cells upon stimulation with dexamethasone. In parallel, the expression of *Scgn* in dexamethasone treated Rin-5F cells was markedly down-regulated. Both mechanisms resulted in a decrease especially of the insulin-granule associated intracellular *Scgn* content in Rin-5F cells under the influence of dexamethasone [16]. At this point, it seems of special interest, that attenuation of intracellular calcium signalling has been hypothesized to underlie the dexamethasone-induced impairment of insulin secretion. Thus, the decreased efficacy of calcium responsiveness following dexamethasone treatment might be at least partly due to the loss of insulin-granule associated *Scgn* and its calcium sensor properties [16].

Clinical applicability of Secretagogin as neuroendocrine marker protein and neurobiochemical serum marker

The neuronal and neuroendocrine-specific expression of *SCGN* is conserved in neoplasias originating from these tissues [6, 17]. Basing on this fact, its reliability as potent marker for the identification of tumours with neuroendocrine differentiation was demonstrated by comparative tissue expression analysis in parallel with the already established neuroendocrine marker proteins chromogranin A, neuron-specific enolase, and synaptophysin [18]. The expression profile of these neuroendocrine marker proteins and of *SCGN* was contrasted by the tissue distribution of a known marker for adenocarcinomas (FK506-binding protein 65). FK506-binding protein 65 was exclusively found in non-neuroendocrine carcinomas. In this line were the results of a proteomics-based study demonstrating marked down-regulation of *SCGN* in colorectal adenocarcinomas when compared with individual-matched normal colorectal derived tissue samples [19]. In normal colorectal mucosa, immunostaining revealed a neuroendocrine cell-characteristic morphology and intra-mucosal location of *SCGN*-expressing cells. The markedly decreased content of *SCGN* in colorectal adenocarcinomas was attributed to the complete absence of this cell type in the majority of the tested carcinoma tissue samples. Remarkably, in a minority of the colorectal adenocarcinomas, which potentially represent a distinct subgroup of this neoplasia, a considerable number of *SCGN* expressing cells was found, [19]. It has to be stressed that results obtained by other groups analyzing the *SCGN* expression in neuroendocrine tumours, in functioning and non-functioning pituitary adenomas as well as its expression in prostate cancer samples in comparison with other neuroendocrine markers underlined the applicability of *SCGN* as neuroendocrine marker protein in routine pathology practice [20-22]. In addition to these clinical applications, *SCGN* represents a reliable marker to confirm the neuroendocrine differentiation of in vitro grown cells. This was demonstrated in studies on the long-term in vitro growth of human insulinoma cells, which revealed that the preservation of insulin-secreting capacities was paralleled by persistent expression of *SCGN* in the in vitro grown human insulinoma cells [23].

Using the monoclonal anti-SCGN antibody mAb D24 (capture antibody) and a highly specific rabbit polyclonal anti-*SCGN* antibody, we established a sandwich capture ELISA for the

detection of *SCGN* in biological fluids [6]. With this newly developed ELISA, the release of *SCGN* from viable Rin-5F cells as well as its solubility in human serum was demonstrated [6, 12]. These facts and the considerable intracerebral *SCGN* expression represented the basis for analyzing the detectability of *SCGN* in human sera following hypoxic neuronal damage associated with clinical symptoms [12]. Remarkably, in none of the sera derived from clinically asymptomatic control subjects detectable amounts of *SCGN* were present. In contrast, the vast majority (90.6%) of patients admitted to the hospital due to neurological symptoms presented with elevated *SCGN* serum levels at least once during the study period of five consecutive days following hospital admission. Patients presenting with transient neurological symptoms had only short periods of detectable *SCGN* serum levels in the peripheral blood, whereas in patients with prolonged or undulating neurological symptoms, *SCGN* was repeatedly detectable on several consecutive days. In these patients, *SCGN* exhibited a characteristic time course, with highest values measured on days 2 and 3 (mean *SCGN* level 20.4 and 19.2 pg/ml, respectively). We compared the releasing pattern of *SCGN* with that of S-100B, an already established serum marker for central nervous tissue damage. This revealed several points of interest: Firstly, S-100B was detectable in all of the analyzed samples, thus confirming the release and detectability of serum markers in association with the occurrence of neurological symptoms in our patients; secondly, the S-100B serum levels exceeded those of *SCGN* by a magnitude of 100x; thirdly, the release pattern of S-100B and *SCGN* differed markedly: whereas the S-100B serum levels where elevated over a period of several days, the *SCGN* serum concentrations reached a peak on days 2 and 3 after admission and rapidly declined thereafter.

CONCLUSION

SCGN is a novel, neuron- and neuroendocrine specific hexa-EF hand protein. Via its calcium-binding properties, it seems to be centrally involved in several calcium dependent processes of excitable cells. Due to its highly specific expression pattern and its serum-detectability, it was additionally established as neurobiochemical tissue and serum marker.

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