

## COMMENT OPEN



# RUNDC3A/SNAP25/Akt signaling mediates tumor progression and chemoresistance in gastric neuroendocrine carcinoma

Ziqi Lin<sup>1</sup> and Hang Fai Kwok<sup>1,2,3</sup>

© The Author(s) 2022

Gastric neuroendocrine carcinoma (GNEC), a heterogeneous group of neuroendocrine neoplasms (NENs) derived from gastric neuroendocrine cells, has been shown to be more aggressive and chemoresistant in gastric cancer, which contributes to the poor prognosis. We analysed transcriptome profiles of tumor/non-tumor tissue from GNEC patients and GNEC cell lines to explore the underlying mechanisms. Our results suggest a critical role for synaptosomal-associated protein 25 kDa (SNAP25) in GNEC. SNAP25 was found to stabilize Akt via modulating its monoubiquitination. We further identified RUN domain containing 3A (RUNDC3A) as an upstream molecule that regulates SNAP25 expression, which is associated with tumor progression and chemoresistance in GNECs. Moreover, these findings were extended into multiple NENs including neuroendocrine carcinomas in the intestinal tract, lungs and pancreas. Identifying the RUNDC3A/SNAP25/Akt axis in NENs may provide a novel insight into the potential therapeutic target for patients with NENs.

*Cell Death and Disease* (2022)13:840; <https://doi.org/10.1038/s41419-022-05294-7>

Neuroendocrine carcinomas (NECs) are currently described as poorly-differentiated neuroendocrine neoplasms (NENs) derived from the diffuse endocrine system and distributed throughout the human body [1]. Accounting for ~4% of all neuroendocrine tumors, gastric neuroendocrine carcinoma (GNEC), a subtype of gastroenteropancreatic neuroendocrine tumors (GEP-NETs), arising from enterochromaffin-like (ECL) cells, is a rare malignant cancer found in the stomach, presenting various characteristics differences from gastric carcinomas (GCs) [2, 3]. With increasing incidence in many countries, GNEC patients had a poorer prognosis than GC patients due to their high malignancy and irresponsiveness to traditional chemotherapy [3]. Therefore, it is crucial and significant to improve the therapeutic effect by identifying the molecular machinery underlying tumor growth and drug resistance in GNEC.

As elucidated in our previous work, we analysed transcriptome profiles of tumor/non-tumor tissue from GNEC patients and GNEC cell lines. A panel of key genes was identified specifically dysregulated in GNEC [4]. Gene Ontology (GO) and pathway enrichment analysis preliminarily revealed a close relationship between synapse-related biological functions and GNEC, and SNAP25 was remarkably involved in these processes [5]. Synaptosomal-associated protein 25 (SNAP25) is an indispensable component of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes which regulate membrane fusion during endocytosis of synaptic vesicle, mostly expressed in neuron-like cells [6]. SNAP25 was found essential in lysosomal trafficking processes and involved in the autophagy pathway, which could subsequently promote SNAP25-expressing epithelial cancers [7]. The expression level of SNAP25 in diffuse

large B-cell lymphoma was correlated with patient survival and prognosis [8]. Although SNAP25 proteins are also abundant in ECL cells [9], whether characteristics of malignant findings and multidrug resistance in GNEC implicate SNAP25 remains unknown, nor has its mechanism been thoroughly explored.

To investigate the role of SNAP25 in GNEC, our recent study was conducted and published in *Cell Death Discovery* [5]. From in vitro and in vivo studies, we observed that SNAP25 knockdown reduced GNEC cells proliferation and tumor growth, as well as increased susceptibility of GNEC cells to multiple chemotherapeutic agents 5-fluorouracil and paclitaxel. Next, we investigated the mechanism by which SNAP25 regulates GNEC. Protein expression analysis showed that the absence of SNAP25 caused a decrease in total Akt protein level and the same reduction in Akt activation (Ser-473). Further results from RT-PCR demonstrated that SNAP25 did not affect Akt mRNA status. While using cycloheximide (CHX) to inhibit protein synthesis, immunoblotting illustrated a longer Akt protein half-life in GNEC cells expressing SNAP25. In addition, in vitro ubiquitination analysis of Akt displayed an elevated monoubiquitination level in GNEC cells after SNAP25 knockdown. Interestingly, we detected a SNAP25-Akt interaction by performing co-immunoprecipitation. These results suggested that SNAP25 might interact with Akt and modulate its monoubiquitination, which could prevent Akt protein from proteasome-dependent degradation in GNEC (Fig. 1).

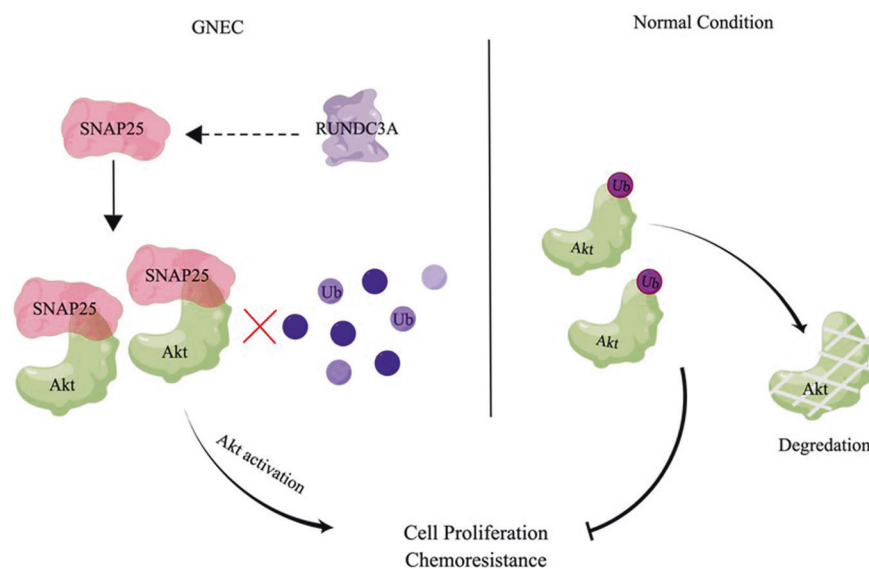
In addition to the stomach, neuroendocrine cells are widely distributed throughout the human body, including the intestinal tract, lungs, and pancreas [10, 11]. To extend our observations to NENs, a comparative analysis of expression profiles was performed

<sup>1</sup>Cancer Centre, Faculty of Health Sciences, University of Macau, Avenida da Universidade, Taipa, Macau SAR. <sup>2</sup>MoE Frontiers Science Center for Precision Oncology, University of Macau, Avenida de Universidade, Taipa, Macau SAR. <sup>3</sup>Department of Biomedical Sciences, Faculty of Health Sciences, University of Macau, Avenida de Universidade, Taipa, Macau SAR. email: hfwkwok@um.edu.mo

Edited by Professor Massimiliano Agostini

Received: 7 July 2022 Revised: 21 September 2022 Accepted: 22 September 2022

Published online: 01 October 2022



**Fig. 1 Schematic representation for the mechanism of RUN Domain Containing 3A (RUNDC3A)/Synaptosomal-Associated Protein 25 kDa (SNAP25)/Akt axis.** The mechanism of RUNDC3A/SNAP25/Akt axis serves as a switch that regulates Akt activation in gastric neuroendocrine carcinoma (GNEC) progression and chemoresistance by stabilizing cytoplasmic Akt. The curve arrows shown in the diagram indicate as positive effect, and the curve T-shaped line indicates as inhibitory effect. See text for further details, as well as the original study by Chen and colleagues [5].

among GNEC and multiple NENs (PNET, SCLC, and SINEC), and a similar panel of synapse-related pathway enrichment was observed. Meanwhile, a protein–protein interaction network analysis presented SNAP25 in the center of other differentially expressed genes, which account for synapsis-related cellular functions. These findings are consistent with early reports that neuroglial synapse-related pathways dysregulation occurs in both primary and metastatic glioma, and their enhancement drives brain tumors progression and induces chemoresistance [12, 13]. Therefore, our data indicated that SNAP25 might play a critical role in synapsis-related cellular functions and the growth of multiple NENs.

According to database analysis, it is also worth noting that RUNDC3A appears to be another common differential expressing genes in NENs. Indeed, we validated that genetic depletion of RUNDC3A significantly reduced GNEC tumor growth both in vitro and in vivo. Given their similar effect on tumor progression, RUNDC3A expression positively correlated with SNAP25 expression in GNEC patients. In addition, a total of lung, pancreas and small intestine NEN datasets were enrolled for similar correlation analysis, and we verified a significant correlation between RUNDC3A and SNAP25 expression in these cohorts. Further proof of western blot identified SNAP25 protein levels were reduced in RUNDC3A knockdown GNEC cells, while regulation of SNAP25 didn't affect RUNDC3A protein abundance. Taken together, these results suggested RUNDC3A is a novel regulator in the upstream of SNAP25-mediated GNEC progression, which might also potentially involve NENs development.

Collectively, our study demonstrated a novel mechanism in which overexpression of RUNDC3A and SNAP25 modulates Akt protein stability to enhance tumor growth and chemoresistance in GNEC. Given the common regulation of SNAP25 and the association between RUNDC3A and SNAP25 in multiple NENs, we conjectured that such mechanism described above is of general applicability. Additionally, considerable research has indicated that PI3K/Akt/mTOR pathway plays a major oncogenic role in many cancer types including neuroendocrine carcinoma [14, 15], which conforms to our observation of activation of Akt in Ser-473 residue. And it is therefore reasonable to speculate that modulation of Akt activity by

RUNDC3A/SNAP25 may have crosstalk with other Akt-related pathways. Further studies are required to verify our hypothesis. From a therapeutic perspective, existing therapy directly targeting Akt and other major components of the pathway has emerged as pharmaceutical imperfection. Our observation of RUNDC3A/SNAP25/Akt axis in NENs progression may provide a novel insight into the potential therapeutic target for patients with NENs.

## REFERENCES

- Oronsky B, Ma PC, Morgensztern D, Carter CA. Nothing but NET: a review of neuroendocrine tumors and carcinomas. *Neoplasia*. 2017;19:991–1002.
- Li T-T. Classification, clinicopathologic features and treatment of gastric neuroendocrine tumors. *WJG*. 2014;20:118.
- Ahmed M. Gastrointestinal neuroendocrine tumors in 2020. *WJGO*. 2020;12:791–807.
- Xie J, Chen P, Xie H, Sun Y, Huang Z, Wei R, et al. Exploration of gastric neuroendocrine carcinoma (GNEC) specific signaling pathways involved in chemoresistance via transcriptome and in vitro analysis. *Comput Struct Biotechnol J*. 2020;18:2610–20.
- Chen P., Wang W., Wong SW, Li J, Wu Q, Zhang S-D, et al. RUNDC3A regulates SNAP25-mediated chemotherapy resistance by binding AKT in gastric neuroendocrine carcinoma (GNEC). *Cell Death Discov*. 2022. <https://doi.org/10.1038/s41420-022-01084-4>.
- Yoon T-Y, Munson M. SNARE complex assembly and disassembly. *Curr Biol*. 2018;28:R397–401.
- Mu Y, Yan X, Li D, Zhao D, Wang L, Wang X, et al. NUPR1 maintains autolysosomal efflux by activating SNAP25 transcription in cancer cells. *Autophagy*. 2018;14:654–70.
- Kobayashi K, Yamaguchi M, Miyazaki K, Imai H, Yokoe K, Ono R, et al. Expressions of SH 3 BP 5, LMO 3, and SNAP 25 in diffuse large B-cell lymphoma cells and their association with clinical features. *Cancer Med*. 2016;5:1802–9.
- Höcker M, John M, Anagnostopoulos J, Buhr HJ, Solimena M, Gasnier B, et al. Molecular dissection of regulated secretory pathways in human gastric enterochromaffin-like cells: an immunohistochemical analysis. *Histochem Cell Biol*. 1999;112:205–14.
- Cives M, Strosberg JR. Gastroenteropancreatic neuroendocrine tumors. *CA Cancer J Clin*. 2018;68:471–87.
- Rekhtman N. Lung neuroendocrine neoplasms: recent progress and persistent challenges. *Mod Pathol*. 2022;35:36–50.
- Weil S, Osswald M, Solecki G, Grosch J, Jung E, Lemke D, et al. Tumor microtubules convey resistance to surgical lesions and chemotherapy in gliomas. *Neuro Oncol*. 2017;19:1316–26.

13. Venkataramani V, Tanev DI, Strahle C, Studier-Fischer A, Fankhauser L, Kessler T, et al. Glutamatergic synaptic input to glioma cells drives brain tumour progression. *Nature*. 2019;573:532–8.
14. Gasparri ML, Besharat ZM, Farooqi AA, Khalid S, Taghavi K, Besharat RA, et al. MiRNAs and their interplay with PI3K/AKT/mTOR pathway in ovarian cancer cells: a potential role in platinum resistance. *J Cancer Res Clin Oncol*. 2018;144:2313–8.
15. Valentino JD, Li J, Zaytseva YY, Mustain WC, Elliott VA, Kim JT, et al. Cotargeting the PI3K and RAS pathways for the treatment of neuroendocrine tumors. *Clin Cancer Res*. 2014;20:1212–22.

### AUTHOR CONTRIBUTIONS

ZL and HFK discussed, conceived, wrote, and revised the paper. HFK supervised the entire project.

### FUNDING

This study was supported by the Science and Technology Development Fund of Macau SAR (FDCT) [0010/2021/AFJ and 0027/2022/A1]. ZL was in receipt of Ph.D. funding from the FDCT.

### COMPETING INTERESTS

The authors declare no competing interests.

### ADDITIONAL INFORMATION

**Correspondence** and requests for materials should be addressed to Hang Fai Kwok.

**Reprints and permission information** is available at <http://www.nature.com/reprints>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2022