

pH and TRAIL receptors interplay in pancreatic ductal adenocarcinoma cells

Dissertation

In fulfillment of the requirements for the degree of Dr. rer. nat. of the
Faculty of Mathematics and Natural Sciences at Kiel University
submitted by

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Kiel, 2022

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Date of the oral examination: 06.02.2023

Parts of this dissertation have already been published in:

Hagelund S, Trauzold A. Impact of Extracellular pH on Apoptotic and Non-Apoptotic TRAIL-Induced Signaling in Pancreatic Ductal Adenocarcinoma Cells. Front Cell Dev Biol. 2022 Feb 24;10:768579. doi: 10.3389/fcell.2022.768579.

Preface

Work presented in this Ph.D. dissertation was conducted from October 2019 to September 2022 and supervised by Professor Anna Trauzold in the Molecular Oncology group, Institute for Experimental Cancer Research, Christian-Albrechts University of Kiel, UKSH Campus Kiel, where the majority of all experiments took place. A minor part of the experiments was obtained from August 2021 to October 2021 under the supervision of Professor Stine Falsig Pedersen, Department of Biology, Section of Cell and Developmental Biology, University of Copenhagen, Denmark.

The overarching aim of this Ph.D. dissertation was, first of all, to investigate the impact of extracellular pH on TRAIL-induced signaling in PDAC and, secondly, how extracellular pH and TRAIL/TRAIL receptors affect intracellular pH.

This Ph.D. was funded and supported by the European Marie Skłodowska-Curie Innovative Training Network (ITN) pH and Ion Transport in Pancreatic Cancer-pHioniC (Grant Agreement number: 813834; H2020-MSCAITN-2018).

Signature: _____

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Kiel, 16.11.2022

Acknowledgments

First and foremost, I would like to thank my supervisor **Prof. Dr. Anna Trauzold**, for giving me the opportunity to carry out my Ph.D. in her group and for letting me research in this exciting scientific field. I am grateful for her always challenging me, her confidence in me, and her enthusiasm for the rest of the group. Her supervision has been thorough and professional and opened my mindset for future challenges.

I would like to extend my gratitude to **Gökhan Alp** for excellent technical assistance and to the whole group of Molecular Oncology in Kiel, hereby **Alshaimaa Adawy, Anna Willms, Anna-Christina Rambow, Carmina Markwardt, Doaa Tawfik, Florian Hoher, Frederik Debus, Frederike David, Hannah Roesler, Insa Aschenbach, and Mouhamad Khouja**, which has provided a positive, welcoming, and friendly working environment. This experience would not have been the same positive one without you. Furthermore, a thank you to **Prof. Dr. Susanne Sebens**, who has always made me and everybody at the Institute feel welcome and comfortable, and to her lab members **Tina Daunke** and **Silje Beckinger** for helping me at the microscope and in sometimes urgent laboratory situations. I would also like to thank **Kirsten Meinhardt** for helping me with organizational and administrative matters.

Additionally, a massive thank you to **Prof. Dr. Stine Falsig Pedersen** as my mentor and during my stay in Copenhagen, Denmark, where she supervised me during this secondment. I am grateful for her expertise and guidance in the pH field and for letting me work with new exciting techniques. Furthermore, I thank her whole lab group, NHE1, especially **Emma Lind Pedersen** and **Tanja Larsen**, for helping, instructing, and bearing with me in this busy period.

During almost my whole time as an international Ph.D. student in Kiel, the COVID-19 pandemic troubled my work and mind. Fortunately, we live in a time when the online world is well-established. This made it possible for me and many others to communicate and present our work anyway and was truly helpful during this special time. I would like to give a special thank you to **Frederike David** and **Hannah Roesler**, also part of the Molecular Oncology group, which have been there through

tough times, taken me on many fun, active and cultural trips, and I also shared cozy dinners with. Without you, I am sure I would not have experienced northern Germany to the same extent.

On a personal level, I wish to thank **my friends and family** for always supporting me and visiting me when possible due to the many COVID-19 restrictions. Especially **my parents**, who were there as soon as the restriction allowed them to visit. I will cherish all the memories I have had.

Finally, I would like to thank the entire **European Marie Skłodowska-Curie Innovative Training Network (ITN) pH and Ion Transport in Pancreatic Cancer-pHioniC** consortium for funding and supporting my Ph.D. I am thankful for the many great, motivating, and inspiring scientific discussions. Sharing this special time with other international students in the same situation as myself was a big help. I am grateful for those physical meetings we could have and for allowing me to visit several exciting destinations.

Zusammenfassung

The author of this dissertation has not translated this abstract.

Das duktale Adenokarzinom des Pankreas (PDAC) ist eine hochgradig lebensbedrohliche Krebsart. Veränderte extrazelluläre und intrazelluläre pH-Werte (pH_e und pH_i) gehören zu den allgemeinen „Hallmarks of cancer“, wobei eine große Bedeutung dem sauren extrazellulärem pH geschenkt wird. Da das PDAC aus dem exokrinen Teil der Bauchspeicheldrüse stammt, besitzt es die Fähigkeit, schwankenden pH-Werten zu widerstehen und diese sogar zu regulieren. Tumor-necrosis-factor (TNF)-related apoptosis-inducing ligand (TRAIL) kann an seine beiden plasmamembrangebundenen Todesrezeptoren TRAIL-R1 und TRAIL-R2 binden und somit Apoptose in Tumorzellen induzieren, während gesunde Zellen verschont bleiben. Dies führte zur Entwicklung von TRAIL/TRAIL-R-basierten Therapeutika. In klinischen Studien blieben die Ergebnisse allerdings hinter den Erwartungen zurück, da Tumorzellen häufig TRAIL-Resistenzmechanismen entwickeln. Dennoch darf TRAIL aufgrund seiner Bedeutung in der Physiologie beziehungsweise Pathophysiologie bei Krebserkrankungen und aufgrund der limitierten Therapieoptionen von Bauchspeicheldrüsenkrebs nicht außer Acht gelassen werden. Aufgrund dessen ist das Ziel dieser Arbeit, das Zusammenspiel zwischen der pH-Mikroumgebung und der TRAIL-induzierten Signalübertragung im PDAC zu verstehen. Um den Einfluss des extrazellulären pHs umfassend zu untersuchen und mögliche kurz- wie langfristige Anpassungen an den pH festzustellen, wurden zwei PDAC Zelllinien Colo357 und Panc1 gewählt. Die beiden Zelllinien sind unterschiedlich sensitiv im Bezug auf TRAIL-induzierte Apoptose. Die Colo357 Zellen sind TRAIL sensitiv und die Panc1 Zellen gehören dem resistenten Typus an. Die langfristige Exposition gegenüber sauren pH_e erhöhte die TRAIL-Sensitivität von Colo357 Zellen, während es bei den Panc1 Zellen kaum einen Einfluss zeigte. Diese PDAC Zellen behielten die TRAIL-Resistenz vermutlich durch (1) einen verringerten Proteinspiegel von TRAIL-R1 und eine geringere Zelloberflächenexpression sowohl von TRAIL-R1 als auch TRAIL-R2 nach der Säureadaption. Ferner (2) konnten in beiden Zelllinien, Unterschiede und Veränderungen in den TRAIL-induzierten pro-apoptotischen und pro-inflammatorischen Signalwegen, sowie in den Expressionslevel der anti-

apoptotischen Proteine beobachtet werden. Des Weiteren (3) konnten säureadaptierte Panc1-Zellen durch Verwendung eines Bcl-xL-Inhibitors (Navitoclax) für TRAIL sensibilisiert werden, was die Bedeutung der mitochondrialen Amplifikationsschleife in (Typ II) PDAC-Zellen hervorhebt. Auch (4) zeigten Panc1 Zellen höhere pH_i -Steady-State-Werte als die Colo357 Zellen, was darauf hindeutet, dass Panc1-Zellen ein robustes ionenregulierendes Netzwerk aufweisen. (5) Erhöhte Konzentrationen des Na^+/H^+ Austauscher 1 (NHE1), Monocarboxylat-Transporter 4 (MCT4) und Carboanhydrase IX (CAIX) nach Säureadaption in Panc1 Zellen verglichen mit den Colo357 Zellen unterstützen diese Hypothese. (6) Es kann angenommen werden, dass die beobachtete Senkung des TRAIL-R1 Spiegels die Wechselwirkungen zwischen TRAIL-R2 und TRAIL-R4 verändert, was wiederum den pH_i -Wert und das Zellüberleben beeinflussen könnte. Weiterhin (7), könnten Säureextruder eine essentielle Rolle bei der Aufrechterhaltung oder Induktion einer TRAIL-Resistenz bei PDAC spielen.

Summary

Pancreatic ductal adenocarcinoma (PDAC) is a highly life-threatening type of cancer. Altered extracellular and intracellular pH (pH_e and pH_i) has become a common hallmark of cancer, with pH_e being particularly acidic. By originating from the exocrine pancreas, PDAC has a natural capacity to withstand and regulate fluctuating pH_e . Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) can bind to its two plasma membrane-bound death receptors, TRAIL-R1 and TRAIL-R2, and induce apoptosis in tumor cells while sparing healthy cells. This led to the development of TRAIL/TRAIL-R-based therapeutic drugs. Unfortunately, clinical trials have shown disappointing outcomes since tumor cells often develop TRAIL resistance mechanisms. Nevertheless, TRAIL cannot be underestimated due to its importance in (patho)physiology and cancer and considering the disappointing therapy options for targeting PDAC. Therefore, this study sought to understand the interplay between the pH microenvironment and TRAIL-induced signaling in PDAC. To extensively study the influence of pH_e , acute and long-term (adaptation) pH_e exposure were projected to the two PDAC cell lines, Colo357 and Panc1. Each has distinct TRAIL sensitivity profiles, Colo357 cells being TRAIL-sensitive and Panc1 TRAIL-resistant. Long-term exposition to acidic pH_e increased TRAIL sensitivity in the TRAIL-sensitive cell line Colo357, whereas Panc1 cells were barely affected. PDAC cells maintained TRAIL resistance presumably through (1) decreased protein levels of TRAIL-R1 and cell surface expression of both TRAIL-R1 and TRAIL-R2 after acid-adaptation. Secondly (2), apparent differences and changes in TRAIL-induced signaling pathways, such as the pro-apoptotic and pro-inflammatory as well as in the expression levels of anti-apoptotic proteins, were observed in the two PDAC cell lines. Thirdly (3), acid-adapted Panc1 cells could be sensitized to TRAIL by using an inhibitor (Navitoclax) of Bcl-xL, proving the importance of the mitochondrial amplification loop in (Type II) PDAC cells. Fourthly (4), Panc1 cells showed higher pH_i steady-state values than Colo357 cells, implying that Panc1 cells exhibit a robust ion-regulating network. (5) Increased Na⁺/H⁺ exchanger 1 (NHE1), monocarboxylate transporter 4 (MCT4), and carbonic anhydrase IX (CAIX) levels in Panc1 cells compared to Colo357 cells upon acid-adaptation supports that hypothesis. (6) Lowering TRAIL-R1 levels can possibly alter interactions between TRAIL-R2 and TRAIL-R4, which may influence pH_i and cell

survival. (7) Acid extruders may be essential in maintaining or inducing TRAIL resistance in PDAC.