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**Area of research:**

The main focus of the working group is the research of new, specific, diagnostic or therapeutic useful potential ‘targets’ of neuroendocrine tumors. Since especially gastroenteropancreatic neuroendocrine neoplasia (GEP-NENs) are extremely heterogeneous in their tumor biology and features, we are interested in this fascinating tumor entity. We also extended our work to neuroendocrine and non-neuroendocrine lung tumor (models) and the interaction with the (cancer related) immune response.

**Background/Rationale:**

Despite there is some progress in the understanding the tumor biology of GEP-NENs, there are still many questions regarding pathogenesis. Often, there are a lot of heterogeneous clinical patterns, which not only differ in localization and functionality of the primary tumor, but also show the main differences in tumor entities (e.g. neuroendocrine tumors of the small intestine). Size of the tumor, ki-67 index or angioinvasion are the parameters which point to the malignancy of each tumor, but it is unexplored which are the underlying mechanisms that lead to such a tumor behavior. We believe that an understanding of underlying signaling pathways is necessary to offer customized therapies to the patient. Sunitinib as a multikinase-inhibitor and Everolimus as a mTOR-inhibitor are fairly new developments for progressive pancreatic NENs (verified investigations of other tumor localizations are on the way for Everolimus), but are possibly limited in their efficacy. We postulate that in this context
‘feedback loops’, which, for example, lead to PI3Kinase/AKT activation after mTOR inhibition, play a significant role. For this reason, it makes sense to have a closer look at modified signaling pathways of specific GEP-NEN-cell lines in the preclinical phase and at the effects of specific inhibitions at these pathways. Furthermore, it would be important to use this gained information to generate new substances, which actually take a specific effect after stratification of the tumors using a molecular biological angle. In this area, we are working close with various pharmaceutical companies that are interested in research moving forward. They supply not only third-party funds, but also interesting substances for preclinical research.

Besides GEP-NENs, we are interested in the bronchial NENs (typical, atypical carcinoid, small and large cell neuroendocrine lung carcinoma), as well as in both entities for the microenvironment, the interaction between tumor cells and stroma and its potential therapeutic impact. At the moment, the latter project is conceived with the research group of Dr. Letsch/Prof. Scheibenbogen, the department of pathology (Prof. Dr. Hummel, Dr. Ruza Arsenic, Dr. Korinna Jöhrens) and our cooperation partners in Bad Berka (ENETS Center of Excellence).

The following goals arise:

The goal of the subordinated projects of the GEP-NENs is to characterize more carefully the specific signaling pathways that are responsible for the malignant transformation of GEP-NENs. Especially mediators, which connect the PI3K-pathway with the expression of survivin respectively regulate survivin in GEP-NENs are to be detected. Most of the therapy approaches of this entity turn out to be difficult or show only effectiveness for a minor collective of patients because of their postulated variegated feedback loops and crosslink of signal pathways. A therapeutic agent, which starts far enough at the bottom of the signal transduction cascade to avoid most of the loops, is of particular interest. For this purpose, a detailed knowledge about the malignancy-associated signal transduction in GEP-NENs is needed. We have identified, in this context, the group of Forkheadbox-proteins as ‘bottleneck-proteins’, which intervene in various signaling pathway cascades and whose inhibition leads to a better therapeutical effectiveness in comparison to the established mTOR-inhibitor Everolimus. We are further interested in understanding the deregulation of tumor suppressor genes upstream of FOXM1, such as Rb and p53 and the role of the proteasome system. The first in vivo experiments were started in cooperation with the University Hospital Freiburg, and further experiments with the Charité – Gastroenterology (CVK) and the University Hospital Hamburg-Eppendorf are planned.

In an affiliated project, neuroendocrine lung tumors shall be examined closer as an independent entity.

Neuroendocrine lung tumors are also a heterogeneous subgroup of the lung carcinomas. This spectrum stretches from clearly differentiated, typical carcinoids with a good prognosis, to moderately differentiated atypical carcinoids to poorly differentiated, highly malignant, small cell lung carcinoma, which have an extraordinarily bad prognosis with mid-survival rates under one year. Because of low incidence, the better differentiated tumors are little investigated, and sufficient differentiation marker are necessary to develop targeted therapy approaches. A biomarker-based differentiation of subtypes is, at the moment, scarcely established, and targeted therapies of these tumor entities are also not yet sufficiently developed. The reason for this is the little data about secretion patterns and little investigated signaling transduction. In this project, most of the focus is kept on the characterization of the signaling pathway of the PI3K-pathway, in comparison between small cell carcinoma of the lung and neuroendocrine lung “carcinoids” to identify possible new ‘targets’ and malignant marker, which help to better classify and distinguish slightly from highly malignant subtypes. This information could lead to a more precise classification, an improved diagnostic and development of new therapeutic approaches. Along the lines of GEP-NENs Forkheadbox-proteins are investigated in neuroendocrine lung tumor (models) as well.
Already ongoing research projects:

Mitose-regulated genes like the so-called 'Chromosomal passenger complex', which consists of aurora kinases, INCENP and survivin and to whom a proliferation promoting function is attributed, are examined by us. Survivin, as a member of the 'inhibitor-of-Apoptosis-Family', is 'bifunctional', anti-apoptotic and mitosis promoting. We have found survivin overexpressed in different gastrointestinal tumors, where we could show that the nuclear expression is of prognostic importance. Survivin could be established as a new relevant prognosis marker, especially for moderately differentiated GEP-NENs (G2), which are, up until now, hardly defined.

We are also interested in the Aurora kinases, further members of the Chromosomal passenger complex. Initially, we have done an immunohistochemical proof of the expression of the Aurora kinases B using our patient collective. The distribution pattern was similar to survivin expression. The commercially available substance ZM 447439, an Aurora kinaseB-inhibitor, was therefore tested in our different gastroenteropancreatic neuroendocrine cell lines, and antiproliferative and proapoptotic effects of different extent were found. This work has already been published.

As a great regulator of Survivin/Aurora kinases we are interested in the Forkhead-box-Protein FoxM1. We could already successfully inhibit FoxM1 in vitro. We are using the gastroenteropancreatic neuroendocrine cell lines BON, QGP-1, KRJ-1 and LCC-18 and prooved the functional importance of FoxM1-Inhibition for apoptosis, cell cycle arrest and growth regulation as well as for the chemotherapeutic re-sensitizing of tumors. For this purpose a combination of experiments with siRNA/pharmaceutical substances and 'established' biochemical therapeutic agents are performed. In parallel, we examine the neuroendocrine lung carcinoma cell lines NCI 727, 720, 810, 460, 2171 and H69. First results are to be published soon.

In cooperation with the Zentralklinik Bad Berka GmbH, ENETs Center of Excellence of neuroendocrine tumors, we are investigating additional probes of patients in terms of proof of survivin in the serum as potential running parameter for these patients. For this, we have gained preoperative and postoperative samples at different time points and during therapy. We also validated the available running parameters of the patients. To prove the qualification of survivin as an intraindividual biomarker parameter, we were also able to use the serum samples of patients from the Charité-CVK, which we correlate with clinical data in long term follow-up (partially > 10 years). This publication is already in preparation.

In cooperation with the clinic of surgery of the Charité-Campus Benjamin Franklin, with PD Dr. M. Kruschewski, we are examining the immunohistochemical expression and the distribution pattern of survivin and Aurora Kinase B, as well as a big collective of colorectal carcinoma of different stages. These examinations are already in the stage of statistical evaluation. In the case of interesting results, cell culture experiments will follow. Not only combination experiments of established chemotherapeutical agents like 5-FU, Oxaliplatin, Irinotecan, but also molecular targeted therapies like Cetuximab or Bevacizumab are noteworthy.

Recent experiments are conducted concerning the interaction between tumor cell signaling and the tumor immune response, which seems to be shut off in many other tumor entities. Since neuroendocrine tumors are only seldom mutated, it seems that there is only rarely an immune response at least in the better differentiated tumors. Here, we use the Array-Technique NanoString nCounter System as well as immunohistochemistry in well and poorly differentiated neuroendocrine neoplasia. First results will be published soon.
Special techniques:
Immunhistochemistry, intracellular colouring for flow cytometry, double staining, SSCP-PCR-Analysis, siRNA-technique, ELISA, immunocytology, Nanostring Technology

Published work:

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<th>Author(s)</th>
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<td>H. Freitag, F. Christen, F. Lewens, I. Grass, F. Briest, S. Iwaszkiewicz, B. Siegmund, P. Grabowski.</td>
<td>Inhibition of mTOR’s Catalytic Site by PKI-587 Is a Promising Therapeutic Option for Gastroenteropancreatic Neuroendocrine Tumor Disease.</td>
<td>Neuroendocrinology, accepted (2016)</td>
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Externally-funded projects:

Ernst-von-Leyden Promotionsstipendium der Berliner Krebsgesellschaft für Inna Georgieva 05/06-05/07
Stipendium des DAAD für Inna Georgieva 05/07-11/07
Sonnenfeld-Stiftung Promotionsstipendium für Inna Georgieva 12/07-03/08
Studentische Forschungsförderung für Yawen Wang 04/08-03/09
Lydia-Rabinowitsch-Stipendium der Charité zur Unterstützung der Projekte von Frau Dr. Patricia Grabowski 04/09-10/09. 10.075 Euro.
Forschungsförderung: “Fox-Proteine und ihre Bedeutung für gastroenteropankratische neuroendokrine Tumore” Partner: Zentralklinik Bad Berka GmbH - Zentrum für neuroendokrine Tumore/TheranosticsResearchNetwork. Geldgeber: Wilhelm und Ingeburg Dinse-Gedächtnisstiftung 03/12-10/12. 18.000 Euro (Personalkosten Franziska Briest)
11/12-12/14 20.000 Euro/Jahr (Sachkosten)
11/12-12/14 Promotionsstipendium für Florentine Lewens (12.000 Euro/Jahr)
11/12-12/14 Personalkosten Franziska Briest (WiMi-Stelle)
2015-2016 10000 Euro/Jahr (Sachkosten)
Lydia-Rabinowitsch-Stipendium der Charité zur Unterstützung der Projekte von Frau PD Dr. Patricia Grabowski 05/12-10/12. 8000 Euro.
Promotionsstipendium der Sonnenfeldstiftung für Frau Helma Freitag, 1000 Euro/Monat (07/13-1/16).
Berliner Krebsgesellschaft: Anschubfinanzierung: “Tumor microenvironment of low and high grade gastroenteropancreatic neuroendocrine neoplasias (GEP-NEN)s: Identification of targets for immune modulation and definition of prognostic and predictive immune biomarkers” (GRFF201505), 40.000 Euro für PD Dr. P. Grabowski/Dr. A. Busse
Sonnenfeld-Stiftung: Tape Station zur Unterstützung des Projektes GFRR201505. 18.000 Euro (2015)
Sonnenfeld-Stiftung: -80°C Tiefkühlgerät zur Unterstützung des Projektes GFRR201505. 13.000 Euro (2016)

Industrial sponsored projects:

1. “Signal transduction pathways in GEP NETs: analysis of associated malignancy markers in vitro and examination of the clinical relevance of potential therapeutic targets in vivo”
   Partner und Geldgeber: IPSEN-Pharma
   Fördersumme: Gesamt 30.500 Euro
2. “Survivin - ein vielversprechender neuer Serumbiomarker bei GEP-NENs”
   Partner und Geldgeber: Novartis Pharma
   Fördersumme: 22.400 Euro
3. Start-up Stipendium zur Etablierung eines deutschlandweiten präklinischen Netzwerkes in neuroendokriner Tumorforschung
   Partner und Geldgeber: Novartis Pharma
   Fördersumme: 14.000 Euro
4. “Lanreotide in bronchial NENs”
   Partner und Geldgeber: IPSEN-Pharma
   Fördersumme: Gesamt 30.000 Euro