Editorial

Intratumoral bacteria as potential contributor of gemcitabine resistance

Melanie S. Patzak, Volker Ellenrieder, Albrecht Neesse

Department Gastroenterology and Gastrointestinal Oncology, University Medical Centre Goettingen, Goettingen, Germany

Correspondence to: Albrecht Neesse, MD, PhD. Department of Gastroenterology and Gastrointestinal Oncology, University Medical Centre Goettingen, Robert-Koch-Str. 40, 37075 Goettingen, Germany. Email: albrecht.neesse@med.uni-goettingen.de.

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers with a 5-year relative survival rate of about 8%. With increasing incidence and death rates pancreatic cancer is projected to become the second leading cause of cancer-related death in the U.S. by 2030 (1,2).

Surgical resection of the tumor with curative intention and adjuvant chemotherapy is possible in early stages of the disease but only available for approximately 15% of pancreatic cancer patients (3). The majority of patients are diagnosed with locally advanced or metastasized tumors. The treatment remains challenging due to the lack of biomarkers, and especially because of its strong resistance to standard chemotherapeutic drugs (4). A hallmark feature of PDAC is the pronounced tumor stroma, which accounts for up to 90% of the tumor mass. Components of this desmoplastic reaction are activated fibroblasts, neural and immune cells, blood vessels and several matricellular proteins. Moreover, secreted components such as hyaluronic acid, collagen, fibronectin and different types of soluble growth factors play important roles in this highly dynamic tumor microenvironment (TME) (5). The impact of the TME on chemotherapeutic resistance is being intensively investigated. In particular, the tumor-stroma crosstalk is described as a central aspect of drug resistance, although the exact interplay of stromal components with neoplastic cells remains largely unknown (5). Recently, stromal depletion approaches have been introduced as a potentially promising novel therapeutic strategy to improve chemotherapeutic drug delivery and response in PDAC. Inhibition of sonic hedgehog (6), hyaluronic acid (7,8) or collagen (9) showed positive effects in preclinical trials. However, clinical trials so far failed to recapitulate preclinical data and resulted in more aggressive and undifferentiated tumors upon fibroblast depletion approaches suggesting that the tumor stroma may also have tumor restraining properties (10,11). Consequently, selective targeting of specific stromal components or stromal reprogramming might be a more powerful and tailored approach (12). In this context, Öhlund *et al.* recently published their work about subpopulations of cancer-associated fibroblasts (CAFs) in pancreatic cancer (13), which further demonstrates the complexity of the TME.

The recently published Science paper by Geller *et al.* (14) brings a new aspect of chemotherapeutic resistance into focus. The main hypothesis of this interesting work is that intratumoral bacteria metabolize and inactivate gemcitabine. The initial and incidental finding of mycoplasma-infected primary cells that conferred resistance to gemcitabine was used to investigate the impact of bacteria on chemotherapeutic resistance. First, the authors demonstrated gemcitabine resistance in a subcutaneous mouse model of colon carcinoma. Here, the authors compared the growth kinetics between tumors established from mycoplasma-positive and mycoplasma-negative tumor cells following gemcitabine therapy. *In vitro* studies using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) revealed significantly decreased levels of native gemcitabine and highly elevated levels of the deaminated inactive metabolite 2', 2'-difluorodeoxyuridine (dFdU) in conditioned medium from the mycoplasma-infected dermal fibroblasts.

The group of Dr. Ravid Straussmann from the

Weizmann Institute of Science, Israel, further extended their studies to 27 bacterial species of which 13 conferred resistance to gemcitabine (14). They hypothesized that expression of bacterial cytidine deaminase (CDD) might be the underlying mechanism of gemcitabine inactivation. The group consequently tested the role of the different isoforms in conferring gemcitabine resistance, and indeed demonstrated the 880-nucleotide long isoform (CDD_L) to be the most crucial isoform in this context. Using the Kyoto Encyclopedia of Genes and Genomes (15) the authors could show that the long isoform is almost exclusively expressed by Gammaproteobacteria. Further *in vitro* experiments convincingly confirmed the role of CDD_L. The ability of the wild-type *Escherichia coli* strain K-12 and CDD knockout strains of this *E. coli* strain in conferring gemcitabine resistance was examined using gemcitabine containing culture media pre-incubated with these bacteria. The bacteria were filtered out and the media transferred to AsPC1 human pancreatic adenocarcinoma cells. As expected, Geller *et al.* could prove gemcitabine sensitivity with the medium from the CDD_L knockout *E. coli* strain. Another colon carcinoma mouse model was used to demonstrate the synergistic effects of gemcitabine and antibiotics *in vivo*. To link their findings to clinical aspects Geller *et al.* examined whether these bacteria are present in the TME of pancreatic cancer patients. Pancreatic cancer was chosen because of the relevance of gemcitabine in the treatment of this devastating disease. Most interestingly, using different methods for the detection of bacterial components like 16S ribosomal DNA, ribosomal RNA as well as bacterial lipopolysaccharides, the authors demonstrated the presence of bacteria in 86 of 113 examined human PDAC samples, whereas bacterial DNA was only present in 3 out of 20 normal pancreas controls. Moreover, the most abundant bacterial species were the Gammaproteobacteria with 51.7% of all reads. Finally, the bacteria were cultured *in vitro* and tested for their ability to confer gemcitabine resistance on RKO and HCT116 human colon carcinoma cell lines. Ninety-three percent of these cultures showed full resistance to gemcitabine.

The importance of the TME in conferring therapeutic resistance is currently intensively investigated in many laboratories around the world. Tumor specific subtypes may help to tailor therapies or provide decision support in a clinical setting concerning the choice and timing of therapies. In analogy to the proposed three epithelial subtypes in pancreatic cancer (16), stroma-specific subtypes have been proposed recently. To this end, Moffitt *et al.* have identified stroma-dependent gene expression signatures, and defined a "normal" and "activated" stroma subtype with independent prognosis (17). The activated pro-inflammatory and macrophage-rich phenotype was characterized by upregulation of a diverse set of genes such as chemokine ligands CCL13, CCL18, gelatinase B, stromelysin 3 and secreted protein acidic and rich in cysteine, thus promoting disease progression (median survival 15 months). Instead, the "normal" stroma subtype was found to highly express pancreatic stellate cell (PSC) markers such as α-smooth muscle actin, vimentin and desmin (median survival 24 months). These findings exemplify the complexity of the TME in PDAC.

In this context, the findings of Geller *et al.* (14) are exciting and novel showing the potential involvement of the microbiome in therapeutic resistance to standard chemotherapies. The future will show whether there is correlation between subtypes and certain bacterial strains, or whether microbiome related subtypes need to be established besides stromal and epithelial subtypes with implications for therapy and prognosis. Targeting gemcitabine resistance by combined treatment with antibiotics would be an attractive and cost-effective approach. A similar drug scavenging effect of the TME has been demonstrated by our group showing that CAFs metabolize and store large amounts of activated gemcitabine intracellularly (18). The proposed mechanism by which CAFs contribute to gemcitabine resistance is similar to the CDD_L metabolizing effect of Gammaproteobacteria. Therefore, combined targeting of several drug scavenging mechanisms in PDAC may further potentiate the effectiveness of standard chemotherapies. However, the drug scavenging hypothesis requires further intensive investigation in preclinical models and patient tissues and should not lead to premature optimism.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7-30.
2. Rahib L, Smith BD, Aizenberg R, et al. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2014;74:2913-21.
3. Stathis A, Moore MJ. Advanced pancreatic carcinoma: current treatment and future challenges. Nat Rev Clin Oncol 2010;7:163-72.
4. Chand S, O'Hayer K, Blanco FF, et al. The Landscape of Pancreatic Cancer Therapeutic Resistance Mechanisms. Int J Biol Sci 2016;12:273-82.
5. Neesse A, Algül H, Tuveson DA, et al. Stromal biology and therapy in pancreatic cancer: a changing paradigm. Gut 2015;64:1476-84.
6. Olive KP, Jacobetz MA, Davidson CJ, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science 2009;324:1457-61.
7. Jacobetz MA, Chan DS, Neesse A, et al. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. Gut 2013;62:112-20.
8. Provenzano PP, Cuevas C, Chang AE, et al. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. Cancer Cell 2012;21:418-29.
9. Chauhan VP, Martin JD, Liu H, et al. Angiotensin inhibition enhances drug delivery and potentiates chemotherapy by decompressing tumour blood vessels. Nat Commun 2013;4:2516.
10. Rhim AD, Oberstein PE, Thomas DH, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell 2014;25:735-47.
11. Özdemir BC, Pentcheva-Hoang T, Carstens JL, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell 2014;25:719-34.
12. Sherman MH, Yu RT, Engle DD, et al. Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. Cell 2014;159:80-93.
13. Öhlund D, Handly-Santana A, Biffi G, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. J Exp Med 2017;214:579-96.
14. Geller LT, Barzily-Rokni M, Danino T, et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science 2017;357:1156-60.
15. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000;28:27-30.
16. Collisson EA, Sadanandam A, Olson P, et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med 2011;17:500-3.
17. Moffitt RA, Marayati R, Flate EL, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet 2015;47:1168-78.
18. Hessmann E, Patzak MS, Klein L, et al. Fibroblast drug scavenging increases intratumoural gemcitabine accumulation in murine pancreas cancer. Gut 2017. [Epub ahead of print].

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