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Original research

Metabolic alteration – Overcoming therapy resistance in gastric cancer via PGK-1 inhibition in a combined therapy with standard chemotherapeutics

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HIGHLIGHTS

- Role of PGK1 in metastatic spread of gastric carcinomas and influence on tumor stem cells.
- Inhibition of PGK1 via adenovirus-shPGK1.
- PGK1-inhibition is able to increase the vulnerability of gastric cancer cells and tumor stem cells.
- PGK1-inhibition may be a new way to overcome the chemotherapeutic therapy resistance.

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ABSTRACT

Background and Objectives: It can be assumed that PGK1 is involved in metastatic spread of gastric carcinomas. Furthermore PGK1 has a proven influence on the characteristics of tumor stem cells. The presence of malignant stem cells, regarding treatment resistance and recurrence, is of considerable importance. We hypothesized that inhibition of PGK1 makes these cells more sensitive to chemotherapeutic agents and therefore mediates an overcome of the existing therapy resistance.
Methods: All investigations were performed with human gastric adenocarcinoma cell lines. Small hairpin RNA knockdown of PGK1 via adenovirus-shPGK1 was used for PGK1-inhibition. Chemotherapeutic agents were 5-FU and mitomycin. FACS, qRT-PCR, and xCELLigence were performed.
Results: Using the medium-sole-control indicating the highest cell viability and Triton indicating the lowest, mitomycin and 5-FU alone showed a significant decrease in cell viability. The treatment with AdvshPGK1 alone already showed a better decrease. The simultaneous application of chemotherapeutics and adenovirus showed the strongest effect and is comparable to the effect of Triton.
Conclusions: We showed a significant decrease in cell viability after the simultaneous application of chemotherapeutics and adenovirus. These results suggest that PGK1-inhibition is able to increase the vulnerability of gastric cancer cells and tumor stem cells to overcome the chemotherapeutic therapy resistance.

1. Introduction

1.1. Epidemiology

Carcinomas of the stomach are globally still the third most common malignant diseases [1,2]. The local variance is very high and especially developing countries display elevated incidence rates. Particularly Germany has a relatively high incidence

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compared with other modern industrialized nations [2]. Here adenocarcinomas of the stomach are the most frequent entity, and with proven association between incidence and increasing age, an increase in mortality due to the local demographic development is to be expected [1]. A major reason for the high mortality is the peritoneal carcinomatosis, which is the most common pattern of metastasis in carcinomas of the stomach [3]. The peritoneal carcinomatosis occurs in 25–30% of patients [4] and has – with a 5 year survival rate of less than 2% – a nearly dismal prognosis [5].

So far, the therapeutic strategy for gastric carcinoma is based primarily on perioperative chemotherapy and complete surgical removal of all tumor tissue. A major factor in the prevention of tumor recurrence is the surgical cytoreduction therapy. Nevertheless, the prognosis of peritoneal carcinomatosis development has not yet been assessed with reasonable certainty [5–9]. There is a clear correlation between a positive intraoperative cytology and an increased risk for the subsequent development of peritoneal carcinomatosis [5,10]. By cytoreductive surgery procedure [11,12] and the combined application of hyperthermic intraoperative chemotherapy (HIPEC) significantly improved long-term results can be achieved, but still, this therapy has to be regarded as palliative [13].

1.2. Phosphoglycerate kinase 1

As part of the question which characteristics enable gastric carcinomas with the same TN-stage and the same grading to cause peritoneal carcinomatosis, microarray studies and real-time PCR analyses could previously show a significantly increased gene expression of phosphoglycerate kinase 1 (PGK1) in patients with peritoneal tumor dissemination [14].

PGK1 is a glycolytic enzyme that provides energy in form of ATP through its catalytic function and thus compensates for the balance of glycolysis. Among others, Otto Warburg stated that metabolic changes and hence glycolysis could be a focus of malignant processes [15]. This view was controversially discussed and considered partly as an epiphenomenon of malignant development [16]. Still, in the following decades, studies could demonstrate that metabolic changes are associated with malignant transformation. Several lines of evidence have been found that inhibition of glycolysis is a way to overcome the drug resistance of certain tumors and preventing tumor metastasis [17,18]. In addition, the depletion of ATP by inhibition of glycolysis could be detected as a successful therapy for the treatment of advanced malignancies in rodents [19]. As a result of the successful use of 18-fluor-2-deoxyglucose-positron-emission-tomography (18FDG-PET) showing an enhanced running glycolysis in malignant cells underlines the fundamental relevance of these processes [20]. We previously could show that PGK1 is promoting tumor progression and metastasis in gastric cancer in a tumor mouse model using positron emission tomography/magnetic resonance imaging [21]. We could also demonstrate a significantly increased incidence of PGK1 in the nucleus of malignant cells which showed a metastatic growth behavior in human gastric carcinomas as well as in tumors of an orthotopic mouse model. This invalidates the perspective of an epiphenomenon for PGK1, since this enzyme, as energy driving enzyme of glycolysis, is active in malignant cells not only in the cytosol, but presumably, with a far more important function namely as a transcription factor, in the nucleus of metastatic cells [22]. Furthermore, PGK1 is associated with the development of breast, pancreatic, prostate and ovarian cancer [23–26], where it is expressed to a greater extent [27,28].

Current research findings could show a new link between cellular metabolism and tumor/progenitor cell differentiation involving PGK1 in the context of metabolic changes which affect cellular differentiation and vulnerability of cells [29]. Thus, it is assumed that this malignant tumor stem cells represent an essential resource for progression, metastasis and recurrence of malignant diseases [30,31]. Meanwhile, this approach had been investigated in a variety of solid tumors and also could be identified in gastric cancer as an important moment for treatment resistance and recurrence [32,33]. Since these cells have a particularly strong resistance to conventional radio- and chemotherapy and continue to favor metastasis and tumor recurrency, they are an important target of novel therapies [34]. In this context, the induction of cellular differentiation of by PGK1 seems a good starting point for new therapeutic treatments.

2. Materials and methods

I Cytotoxicity tests of standard chemotherapeutic agents 5-FU and mitomycin C to determine the optimum concentration for the inhibition of tumor cells.

2.1. Cell Culture

The human gastric adenocarcinoma cell line 23132/87 (ACC409) was purchased from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) and cultured according to manufacturer's instructions in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) (Lonza, Basel, Switzerland) at 37 °C in a 5% CO_2 incubator.

2.2. Real-time cell proliferation assay: xCELLigence SP system

The human gastric carcinoma 23132/87 cells (12.5×10^3 cells/well) were seeded in 96-well plates (E-Plate 96, Roche Applied Science, Mannheim, Germany). Cells were monitored by measurements of electrical impedance in 30 min intervals using the xCELLigence SP system (Roche Applied Science) [35]. Cell index values were calculated and all curves normalized using the RTCA Software 1.2. Cells were treated with different concentrations of mitomycin C and 5-FU 24 h after seeding and monitored for additional 96 h (Fig. 1).

II Transient transduction with adenoviral vector shPGK1 – functionality test

Small hairpin RNA knockdown of PGK1 (shPGK1) via Adv-shPGK1.

The above mentioned cells were incubated with an adenovirus (Sirion Biotech, Martinsried, Germany) containing gfp-marked shPGK1.

(50-CATACCTGCTGGCTGGATGG-30 (forward),
50-CCCACAGGACCATTCCACAC-30 (reverse))

with multiplicity of infection (MOI) ratios of 200, 300 and 500 for 48 h to silence the PGK1 expression. The adenovirus without shPKG1 was used as a negative control.

2.3. Fluorescence-activated cell sorting (FACS)

After 48 h a fluorescence-activated cell sorting (FACS) analysis detecting the GFP signal was performed at Flow Cytometry Core Facility at university clinic Tuebingen (Fig. 2).

2.4. Quantitative real-time-PCR (qTR-PCR)

After 72 h a quantitative real-time-PCR (qRT-PCR) was