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Original Article

Therapeutic effects of Argyrin F in pancreatic adenocarcinoma

Xi Chen [a], Khac Cuong Bui [a], Samarpita Barat [a], Mai Ly Thi Nguyen [a], Przemyslaw Bozko [a], Bence Sipos [b], Markus Kalesse [c], Nisar P. Malek [a], Ruben R. Plentz [a, *]

[a] Department of Internal Medicine I, Medical University Hospital, Tübingen, Germany
[b] Institute of Pathology, University of Tübingen, Germany
[c] Institute of Organic Chemistry & Centre of Biomolecular Drug Research, Leibniz University of Hannover, Germany

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease with limited treatment options. The proteasome inhibitor Argyrin A, a cyclic peptide derived from the myxobacterium *Archangium gephyra*, shows antitumoral activities. We hypothesize that his analogue Argyrin F (AF) may also prevent PDAC progression. We have used PDAC cells and engineered mice (Pdx1-Cre; LSL-KrasG12D; p53 $^{lox/+}$) to assess AF anticancer activity. We analyzed the effect of AF on proliferation and epithelial plasticity using MTT-, wound healing-, invasion-, colony formation-, apoptosis-, cell cycle- and senescence assays. *In vivo* treatment with AF, Gemcitabine (G) and combinational treatment (AF + G) was performed for survival analysis. AF inhibited cell proliferation, migration, invasion and colony formation *in vitro*. AF impaired epithelial-mesenchymal transition (EMT), caused considerable apoptosis and senescence in a dose- and time-dependent manner and affected cell cycle G_1/S phase transition. G treatment achieved longest mice survival, followed by AF + G and AF compared to vehicle group. However, AF + G treatment induced the largest reduction in tumor spread and ascites. In conclusion, we have demonstrated that AF prevents PDAC progression and that combined therapy was superior to AF monotherapy. Therefore, AF treatment might be useful as an additional therapy for PDAC.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease with poor prognosis [1]. Surgery is the only curative therapy, but many patients present at diagnosis with advanced tumor stage. Patients with metastatic disease have a median survival of only 6 months [1–3]. Palliative chemotherapies with FOLFIRINOX or nab-Paclitaxel + Gemcitabine (G) are recommend as first line chemotherapy for patients with non-resectable PDAC [2,3], but more drugs are urgently needed to improve overall survival and provide new systemic therapeutic options.

It was shown that Argyrin A, a cyclical peptide derived from the myxobacterium *Archangium gephyra* has antitumoral activities [4]. Argyrin A acts by inhibition of 20S proteasome that leads to the stabilization of different proteasome substrates. Moreover, it was shown that one of the proteasome substrates namely $p27^{kip1}$ plays a critical role in the antitumor activity of Argyrin A. Moreover, patterns of Argyrin A analogues were synthesized or isolated from natural sources. One of these analogues, Argyrin F (AF) has similar biological activity as Argyrin A, however shows better solubility in water-based solutions (Fig. 1A) [5].

In 2012 two independent studies by Bielecki et al. [6] and Nyfeler et al. [7] mentioned a critical role of mitochondrial translational machinery in response to the Argyrin family. Here, we report for the first time about the application of AF in an *in vitro* and *in vivo* model of PDAC carcinogenesis. We show that AF treatment inhibits cell proliferation, migration, invasion and colony formation by partial induction of apoptosis and epithelial-mesenchymal transition (EMT). We further demonstrated that administration of AF in combination with G caused most significant PDAC reduction by inhibiting cell proliferation and tumor angiogenesis. Thus, our findings support AF as a new systemic treatment option for PDAC.

Materials and methods

Cell culture

Human pancreatic cancer cell lines Panc-1 and KP3 were obtained from the laboratory of Nabeel Bardeesy, MGH Cancer Center, Boston, USA. Cell lines were

* Corresponding author. Department of Internal Medicine I, Medical University Hospital, Otfried-Müller-Str. 10, 72076 Tübingen, Germany. Fax: +49 7071 29 5906.
E-mail address: Ruben.Plentz@med.uni-tuebingen.de (R.R. Plentz).

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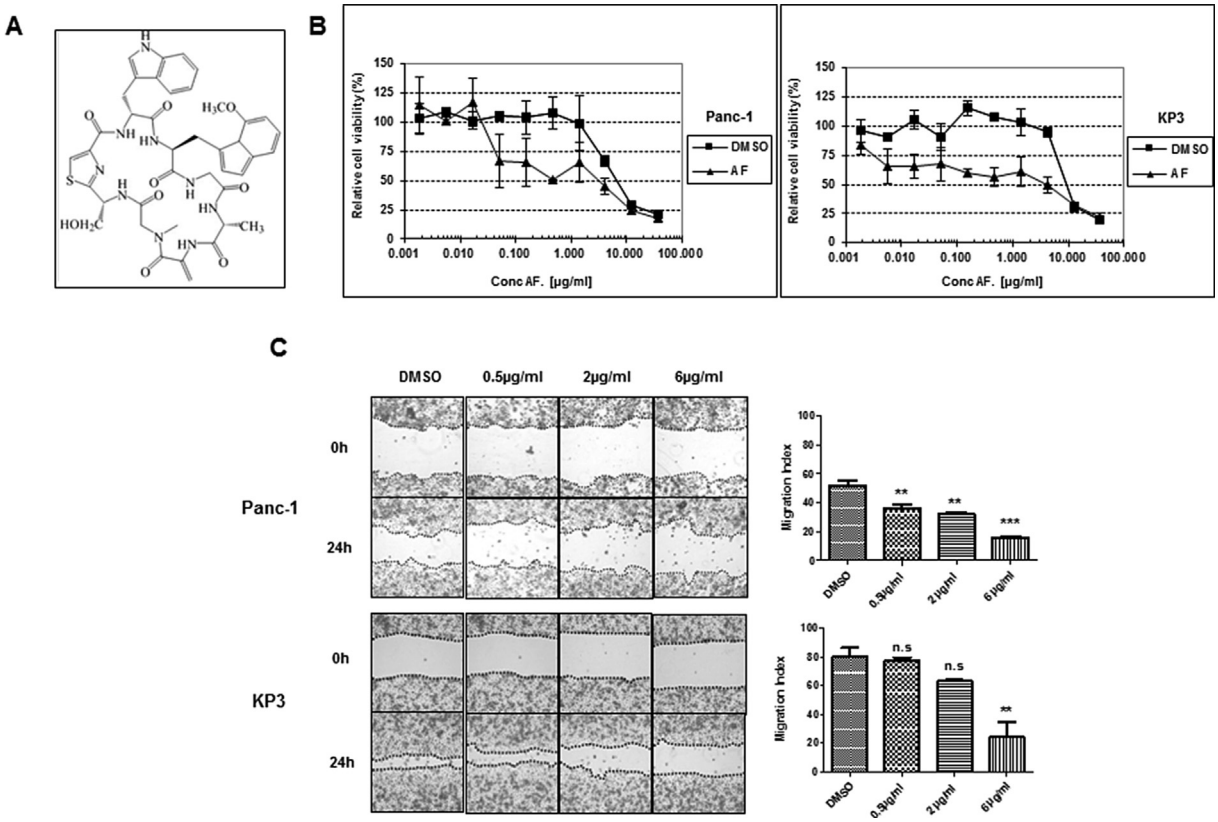

Fig. 1. AF treatment inhibits proliferation and migration of human PDAC cell lines. A) Chemical structure of Argyrin F (AF). B) Cytotoxic effect of AF (IC_{50}): Panc-1 and KP3 cells were treated for 72 h with increasing concentrations of AF (0,001 µg/ml to 100 µg/ml). Cell viability was measured using a cell proliferation assay, DMSO was used as a control. C) Wound healing experiments were done for Panc-1 and KP3 cells cultured with DMSO and AF (0.5 µg/ml, 2 µg/ml and 6 µg/ml). The dotted lines are representing the edges of the wound. Photographs were taken at 0 and 24 h under light microscope (40× magnification). The migration index was calculated as described in Materials and methods and plotted in bar graphs. Differences were considered as statistically significant when the *P*-value was <0.001 (***), <0.01 (**) or not significant "n.s." The error bar represents standard deviation.

maintained at 37 °C under a 5% CO2 environment in RPMI 1640 + L-Glutamine + 25 mM HEPES (Invitrogen, Karlsruhe, Germany) enriched with 10% fetal calf serum (FCS) (Biochrom, Berlin, Germany) and antibiotics of penicillin/streptomycin (100 units/ml) (Invitrogen, Karlsruhe, Germany).

Drugs and treatment

AF was supplied by the Institute of Organic Chemistry & Centre of Biomolecular Drug Research, Leibniz University, Hannover, Germany. In a 4-week toxicity study, AF was administered every 3rd day (on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27; total 14 treatments) by intravenous bolus administration into the tail vein of SPF-bred Wistar rats of both sexes at dose levels of 1.25 mg/kg, 2.5 mg/kg or 5.0 mg/kg body weight. No test item-related (AF) mortality or clinical signs were observed in animals treated with 5.0 mg/kg (AF). AF was prepared as a 5 mg/ml stock in dimethylsulfoxide (DMSO) (AppliChem, Darmstadt, Germany). The drug was divided in aliquots (0.5 mg/ml), frozen at −20 °C and used for *in vitro* and *in vivo* experiments. Cells were treated with DMSO or AF in different concentrations (0.5 µg/ml, 2 µg/ml, 6 µg/ml) and were analyzed after 24 h, 48 h and 96 h for certain experiment.

Cell proliferation assay, apoptosis assay, migration assay, invasion assay, soft agar assay, cell cycle analysis and Western Blot analysis

The protocols used in this study are described in Supplementary Materials and Methods.

Animals and treatment

Transgenic Pdx1-Cre mice and p53 $^{lox/+}$ mice were obtained from Aram Hezel (University of Rochester Medical Center, USA). Kras mice were kindly provided by Lars Zender (UKT, Germany). Mice were crossed and genotyped to obtain animals with the following background: Pdx1-Cre; LSL-Kras^{G12D}; p53 $^{lox/+}$ [8]. 6 mice were treated intraperitoneally with AF (1 mg/kg/body weight, on day 1–day 3), 5 mice were treated with Gemcitabine (Eli Lilly and Company) (100 mg/kg/body weight, once per week), 6 mice were treated with a combination of AF (1 mg/kg/body weight, on day 1–day 3) + G (100 mg/kg/body weight, on day 5) and 6 mice were treated with vehicle (PBS) (Thermo Fisher Scientific). Treatment was started at 14 weeks of age and mice were sacrificed when critical illness was developed. All treatment solutions were prepared fresh on the day of delivery. Health status of mice was inspected every day. Sizes of pancreas and visible tumors were measured by caliper and organ tissues were harvested. Tumor volume was calculated using the following formula: Tumor volume V = [(∂\6) × (Length) × (Width^2)]. Tumor tissues were snap frozen for protein analysis and fixed in 4% formalin for histology. All animals were analyzed for liver metastasis and ascites (Table 1).

Ethics statement

Mice used in this study were maintained in the animal care of Medizinische Universitätsklinik Klinik Tübingen (UKT), Germany. All experimental protocols were reviewed and approved by institutional guidelines for animal care of UKT and Baden Württemberg (protocol no: M11/13), and all studies were performed according to the methods approved in the protocol.

Histology and immunoblotting

Detailed information about antibodies and protocols can be found in the Supplementary Materials and Methods.

Statistical analysis

All the experiments were repeated 2–3 times. The results were analyzed using software Graphpad prism version 5.0 (GraphPad Software, San Diego, USA) and SPSS

Table 1
Liver metastasis and ascites in KPC mice.

	Vehicle (n = 6)	AF (n = 6)	G(n = 5)	AF + G (n = 6)
Liver metastasis	66.7%	16.7%	20%	0%
Ascites	100%	66.7%	20%	16.7%

AF = Argyrin F; G = Gemcitabine; AF + G = Argyrin F + Gemcitabine.

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