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RESEARCH PAPER

Suppression of lung metastases by the CD26/DPP4 inhibitor Vildagliptin in mice

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Abstract Metastases rather than primary cancers determine nowadays the survival of patients. One of the most common primary malignancies is colorectal cancer and this type of tumor is characterized by a high tendency to spread metastases to the lung and liver. CD26/DPP4 is a transmembrane molecule with enzymatic functions which cleaves biologically active peptides. Recently, CD26/DPP4 has become the focus of cancer research and it was shown that CD26/DPP4-positive cancer cells display increased metastatic activity. Here, we tested if the CD26/DPP4-inhibitor Vildagliptin suppresses the development and growth of mouse colorectal lung metastases. This inhibitor of CD26/DPP4 was employed on mouse (C57BL/6) colorectal lung metastases, established by intravenous injection of the syngeneic cell line MC38. For mechanistic analysis, a subcutaneous tumor model was used. The treatment with Vildagliptin significantly suppressed both, the incidence and growth of lung metastases. Autophagy markers (LC3, p62, and ATF4) decreased, apoptosis increased (TUNEL, pH3/Ki-76), and the cell cycle regulator pCDC2 was inhibited. In conclusion, we here showed an anti-tumor effect of Vildagliptin via downregulation of autophagy resulting in increased apoptosis and modulation of the cell cycle. We therefore propose Vildagliptin for the evaluation as a new therapeutic approach for the treatment of colorectal cancer lung metastases.

Keywords CD26/DPP4 · Lung metastases · Autophagy · Apoptosis

Introduction

The lung is one of the major sites of metastasis from colorectal cancer [1]. Colorectal cancer has a high tendency to spread metastases with the second highest mortality in Europe [2]. While primary cancers are under improved control by multimodal therapy, the development of metastases is considered the most important factor for the patient's survival. If untreated, patients suffering from colorectal lung metastases have a median of 10 months survival [3].

CD26/DPP4 is a multifunctional transmembrane glycoprotein constitutively expressed on many cell types and within body fluids in a soluble form [4]. CD26/DPP4 comprises an exopeptidase enzymatic activity and cleaves off N-terminal dipeptides preferentially after Ala or Pro [5]. Furthermore, CD26/DPP4 can associate with fibroblast activation protein (FAP) and binds to extracellular matrix collagen and fibronectin [6]. During the last decade, diverse scientific disciplines have focused on CD26/DPP4, describing its involvement in the fields of immunology,

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diabetology, cardiology and cancer [7]. The unique enzyme-substrate specifications of CD26/DPP4 render several key peptides susceptible to catalytic cleavage which modulates their biological effects. The development of potent CD26/DPP4-inhibitors has led to the identification of CD26/DPP4 as a target for the successful treatment of diabetes. Currently, various commercially available CD26/DPP4-inhibitors including Vildagliptin (which we employed in this study) are routinely used in clinical practice. Remarkably, only mild side effects from the treatment with these CD26/DPP4-inhibitors have been observed up to the present [8].

A role of CD26/DPP4 in tumor biology has been suggested according to its functional and enzymatic properties in T cell lymphoma, mesothelioma, melanoma, renal carcinoma, colorectal cancer, and lung cancer [9–15]. Recently, a CD26/DPP4 antibody was employed as a therapeutic measure in mesothelioma and T-cell lymphoma showing down-regulation of RPB1 followed by inhibitory DNA binding of CD26/DPP4 [16–18]. In a rat study, the effect of CD26/DPP4-inhibition on colon carcinogenesis was shown by a long term treatment with the CD26/DPP4-inhibitor Sitagliptin [19]. These data emphasizes the involvement of CD26/DPP4 in cancer biology.

By using an established orthotopic/simultaneous iv.-injection model of syngeneic tumor development [20–22], we report here (i) an in vitro mechanism of tumor growth inhibition related to cell cycle, (ii) the prevention of metastasis and inhibition of metastases growth from colorectal cancer by Vildagliptin in a syngeneic cell line-induced lung tumor model, and (iii) the regulation of tumor autophagy by Vildagliptin treatment, resulting in apoptosis and less cell proliferation. As Vildagliptin is in safe and effective clinical use for diabetic disease, this therapeutic approach could be a novel concept in the treatment of lung metatastic disease.

Materials and methods

Animal care

Male wild type (C57BL/6 Charles River, Germany) mice were used for all experiments ($n > 5$). Breeders of CD26/DPP4$^{-/-}$ mouse (based on C57BL/6 strain) were obtained from the European Mouse Mutant Archive (Orleans, France) and maintained in the Biological Central Labor, University Hospital Zurich. Animals were fed a standard laboratory diet with water and food ad libitum and were kept under constant environmental conditions. All experimental procedures were approved by the Swiss animal welfare authorities and performed in accordance with the institutional animal care guidelines.

Lung metastasis mouse model

Green fluorescence protein (GFP) labelled syngeneic mouse colorectal cancer cells (MC38: 100×10^3 cells/g mouse) were prepared in serum free DMEM (100 µl) and injected into the inferior vena cava after middle line laparotomy under isoflurane anesthesia. After checking relevant vital signs (e.g. respiration rate, organ color) and integrity of vasculature at injection site, the abdominal wall was closed by a running suture. Vildagliptin was administered via two different routes in order to test the effect as a preventive treatment against lung metastases of colorectal cancer (pre-treatment) or a treatment for the developed lung metastases of colorectal cancer (post-treatment). Prior to injection of tumor cells (MC38, 100×10^3 cells/g mouse), subcutaneous pre-treatment of Vildagliptin was performed for 3 days (100 mg/kg) to test the incidence of metastasis (Suppl. 1a). Post-treatment was performed by adding Vildagliptin into drinking water ($\sim$40 mg/kg/day) 10 days after tumor cell injection in order to inhibit the growth of established tumors (Suppl. 1b). Three weeks after tumor cell injection, total lungs were weighed and homogenized for quantification of GFP labeled tumor cells. Animals were sacrificed by exsanguination followed by flushing with saline and *en bloc* resection of thoracic organs including bilateral lungs, heart, and thymus. The whole lung was frozen for the quantification of tumor load, and heparinized plasma was collected for the measurement of CD26/DPP4 activity.

Tumor cell lines

NCI-60 cell lines (A549, H460, and Ekvx) were obtained from Charles River (Boston, USA) under material transfer agreement with the National Cancer Institute (Bethesda, USA). HT29, LLC and CT26 cell lines were purchased from American Type Culture Collection (Manassas, USA). Authenticated cell lines by both providers were stored at early passages (<3) in liquid nitrogen and were used in the experiments for no more than 6 months. MC38 and GRX cell lines were kind gifts of Dr. Lubor Borsig and Dr. Radovan Borojevic, respectively. Both cell lines were stored in liquid nitrogen and were used in the experiment no more than 6 months. All cell lines were cultivated in DMEM containing 10 % FBS and penicillin/streptomycin within a 5 % CO_2 chamber.

Subcutaneous tumor model

We employed the model of subcutaneous (sc.) tumor development by injection of the cell line sc. in order to prove the effects and mechanisms of this study how the growth of metastases is inhibited (post-treatment

iv. model). Cell lines (MC38) (1×10^6 cells/mouse) were injected under the skin of mice (back) in serum free DMEM. Vildagliptin was given in drinking water (0.2 mg/ml) for 2 weeks. Following exsanguination, the tumor was isolated from skin and weighed.

DPP4 assay

CD26/DPP4 enzymatic activity was analyzed in mouse plasma using glycyl-prolyl-4-methoxy-β-naphthylamide (Gly-Pro-4-Me-β-NA) as a fluorogenic substrate as described previously [23]. Briefly, in a 96-well plate, 10-µl samples were mixed with 0.5 mM Gly-Pro-4-Me-β-NA in 50 mM Tris buffer, pH 8.3, in a final volume of 110 µl. CD26/DPP4 activity was determined kinetically for 10 min at 37 °C by measuring the velocities of 4-Me-β-NA release (λex = 340 nm, λem = 430 nm) from the substrate (all reagents are from Sigma-Aldrich, Germany).

In vitro experiments

Both mouse and human cell lines were maintained in DMEM containing 10 % FBS, penicillin/streptomycin. The cell lines were seeded into 24-well plates to reach 70 % confluence. Two days after cell seeding, media were exchanged with serum-free one. Twelve hours later, cells were exposed according to the experimental conditions. The treatment was performed after dilution of stock solutions including Vildagliptin, Cisplatin, Gefitinib, 3 methyladenine (3MA) (Sigma-Aldrich), SP600125 (Tocris) by serum free media (DMEM). Daily metabolic activity was assessed by the methylthiazolyldiphenyl-tetrazolium bromide (MTT, Sigma-Aldrich) assay. For western blotting assays, cell lines were prepared in 10 cm dishes. These experiments were triplicated.

Protein expression analysis

Samples were homogenized in lysis buffer containing a protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany), phosphatase inhibitor cocktail 3, 50 mM Tris, 150 mM NaCl, 5 mM EDTA, and 0.5 % NP-40 (Sigma-Aldrich). The protein concentration was determined using the Bradford protein assay (BioRad, Hercules, USA). Reducing SDS-PAGE was performed and samples were blotted onto a PVDF-membrane. p62 (MBL), CDC2, pCDC2, PTEN, Cleaved caspase3, LC3, ATF4, AKT, pAKT (Cell signaling), E-cadherin, N-cadherin, Vimentin (Abcam) and ID1 [24] antibodies were tested. Loading control was a rabbit anti-GAPDH (Abcam) and anti-beta actin (Sigma-Aldrich). Secondary antibody binding and detection was performed according to standard protocols with the ECL detection reagent (BioRad).

Histology

Formalin fixed and paraffin embedded samples were stained by antibodies against Ki67, p-Histon3, and GFP. TUNEL stain was used for detection of apoptotic cells within the tumor.

Statistical analysis

Data were presented as mean ± SD. Groups were compared with the Student t-test for unpaired samples using Prism 4.0 (GraphPad Software, San Diego, CA, USA). A two-sided p value < 0.05 was considered to be statistically significant.

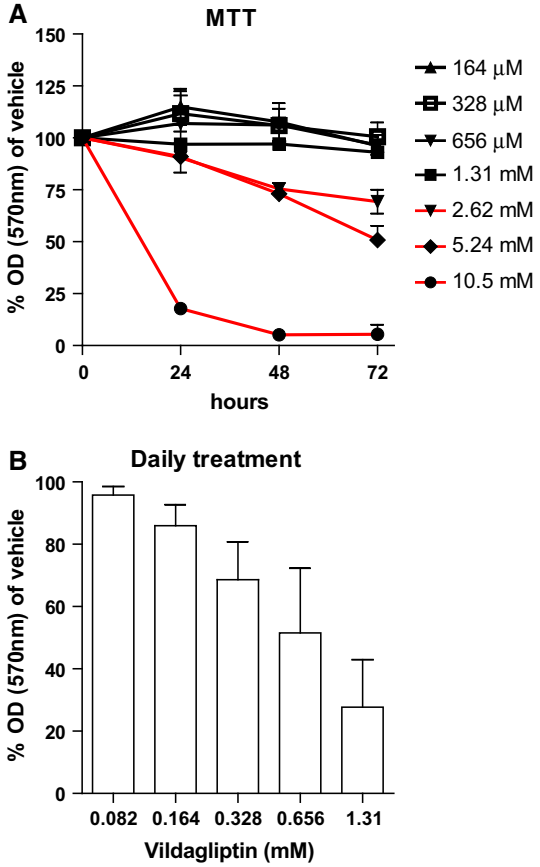

Fig. 1 In vitro cytotoxicity test of Vildagliptin. Various doses (164 µM—10.5 mM) of Vildagliptin were administered to the MC38 cell line grown in 24 well plates to test the metabolic activity of tumor cells. The metabolic activity was shown by MTT assay measured at 24, 48, 72 h (**A**), and 7 days (**B**). Vildagliptin was given one time after 12 h of serum free medium to test the cytotoxicity during 3 days (**A**). The treatment of Vildagliptin showed a pronounced cytotoxicity in a dose-dependent manner, also confirmed in other cell lines (Suppl. 2). Similar to the single treatment with high doses, continued daily treatment with non-cytotoxic doses (0.082–1.31 mM) (in vivo-situation) of Vildagliptin reduced the metabolic activity of the tumor cell line MC38 in vitro (**B**)

Results

Cytotoxicity test of Vildagliptin in tumor cell lines

To test the effect of Vildagliptin directly on tumor cells first in vitro, we tested different doses (from < 164 µM to 10.5 mM) (Fig. 1A) on various cell lines with existing resistance to conventional chemotherapeutics (cisplatin and gefitinib) (Suppl. 2a). The IC50 for MC38 cell line (mouse colorectal cancer) after 3 days was 5.24 mM (Fig. 1A). Regardless of critical mutations of *KRAS*, *EGFR*, and *BRAF* in those cell lines (Suppl. Table 1), Vildagliptin treatment showed a cytotoxicity in a dose-dependent manner. To mimic the in vivo situation, we tested daily Vildagliptin treatment for 7 days by giving non-cytotoxic doses similar to the dose used in the in vivo experiments (40 mg/kg/day). Interestingly, these non-cytotoxic doses of Vildagliptin (doses below 2.62 mM, see Fig. 1A) decreased the metabolic activity of MC38 cell line in vitro (Fig. 1B). This effect could be confirmed in various mouse and human cell lines (Suppl. 2). Taken together, we found a cytotoxic effect of Vildagliptin in tumor cell lines as well as after repeated treatment of non-cytotoxic doses of Vildagliptin, mimicking the in vivo situation. However, of note, there is still the possibility that under these high concentrations of Vildagliptin, also cytoplasmatic enzymes are prone to inhibition by Vildagliptin.

Vildagliptin suppresses the incidence of colorectal lung metastases in mice

Next, we aimed to prevent the development of lung metastases of colorectal cancer cells in mice by the pre-treatment with Vildagliptin. Vildagliptin (100 mg/kg) or saline (vehicle) was administered for 3 days by subcutaneous injection into mice before the injection of the syngeneic cell line (MC38). The cell line (100×10^3 cells/g mouse) was intravenously injected into the inferior vena cava of C57BL/6 and CD26$^{-/-}$ mice and harvested 1 day or three weeks after injection in order to test the metastatic activity of colorectal cancer cells. The cell line injection was performed 3 h after the last administration of Vildagliptin to obtain an optimal effect of the treatment towards the injected cells (Fig. 2A). One day after injection,

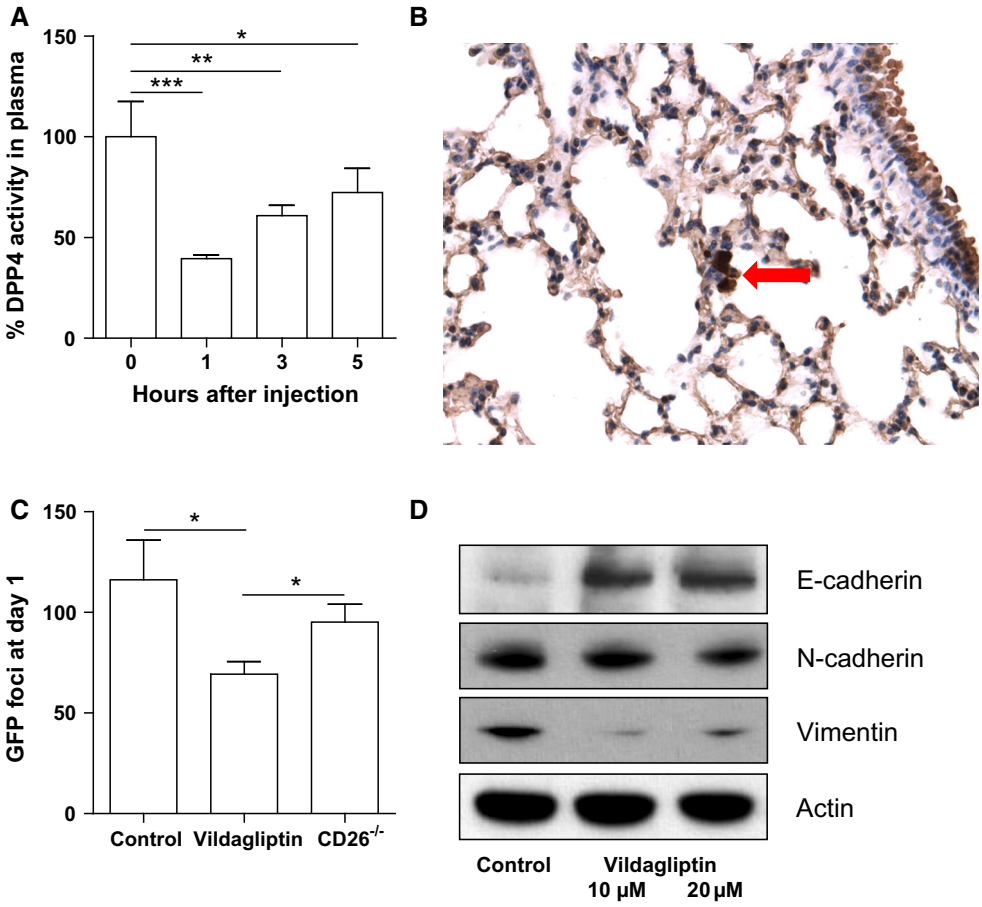

Fig. 2 Prevention of lung metastases by Vildagliptin pre-treatment in the MC38 cell line. Prior to injection of tumor cells (MC38, 100×10^3 cells/g mouse), subcutaneous pre-treatment of Vildagliptin was performed for 3 days (100 mg/kg) in order to test the incidence of metastasis. The CD26/DPP4 activity was measured serially 1, 3, and 5 h after Vildagliptin treatment (100 mg/kg) (**A**) (n = 4). The immunohistochemistry of GFP showed tumor cell foci one day after injection (*arrow*) (**B**), counted histologically (×200) through all lobes of lungs from control, pre-treatment of Vildagliptin, and CD26$^{-/-}$ mice (n = 3) (**C**). EMT markers were significantly modulated by the pre-treatment of Vildagliptin in vitro (**D**) (Suppl. 3a–c) (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

metastatic tumor cells were counted under the microscope (×200) throughout all lobes of the lung (Fig. 2B, C). Here, pretreatment of Vildagliptin significantly decreased metastases. However, compared to controls, $CD26^{-/-}$ mice showed a similar number of metastases. Of note, the in vitro treatment of low doses of Vildagliptin to the MC38 cell line significantly decreased markers of EMT (Fig. 2D, Suppl. 3a–c).

In order to quantify the tumor that had grown within the lung for three weeks, we homogenized the total lung and measured the fluorescence (Excitation 485 nm and Emission 508 nm) (Fig. 3A). Tumor size estimated by fluorescence was significantly reduced by Vildagliptin pretreatment which correlated with the macroscopic observation (Fig. 3C) and the wet weight of total lung (Suppl. 3). Collectively, we found that pre-treatment of tumors with Vildagliptin prevents the incidence of colorectal cancer metastases in the lungs.

Vildagliptin suppresses the growth of lung metastases in mice

In order to test the effect of Vildagliptin against an established tumor in the lung, we induced lung tumor - growth by intravenous injection of syngeneic cell line (MC38) as the metastasis model and started post-treatment of Vildagliptin (~40 mg/kg/day in drinking water) 10 days after cell line injection. Three weeks after cell line injection, we harvested the tumor developed in the lungs. Measured GFP and total lung weight were significantly reduced by post-treatment with Vildagliptin (Fig. 3B, C). This inhibitory effect of Vildagliptin was confirmed by testing it in the subcutaneous tumor model (Fig. 4A): TUNEL positive cells were significantly increased in Vildagliptin-treated tumors compared to controls (Fig. 4B–D). In line with the growth of metastases, the size of the subcutaneously developed tumor

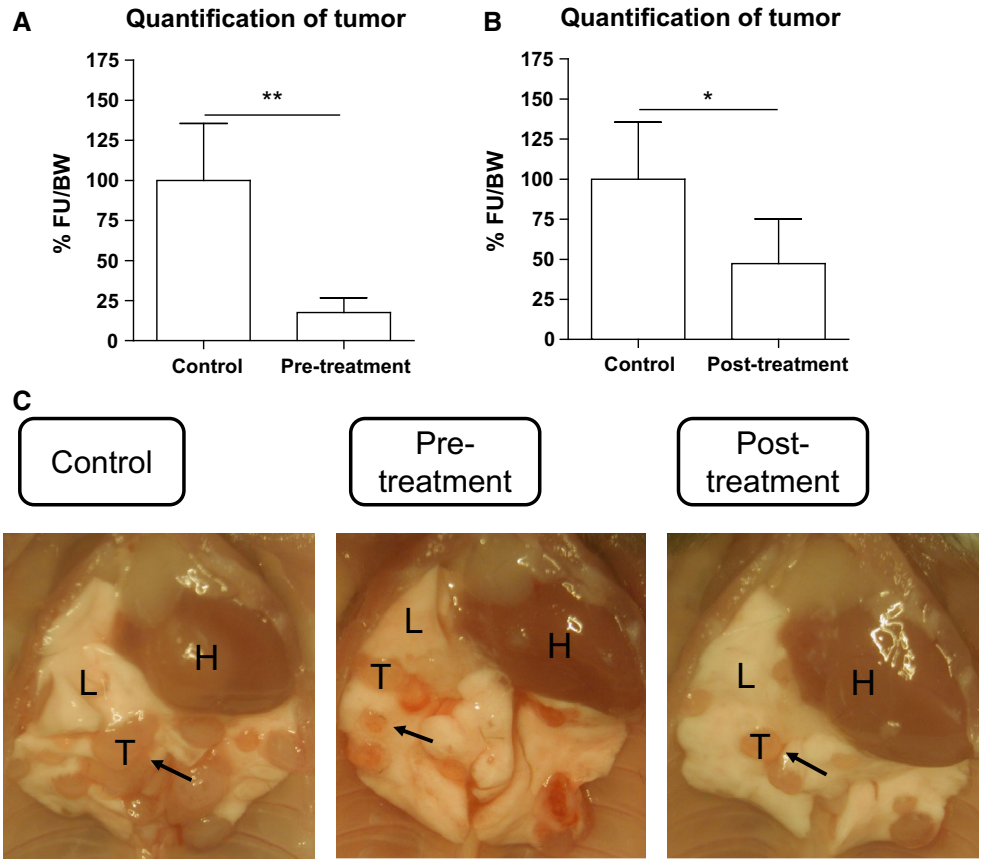

Fig. 3 Vildagliptin treatment reduced lung metastases growth. Post-treatment was performed by administering Vildagliptin into drinking water (~40 mg/kg/day) 10 days after tumor cell injection in order to inhibit the established tumor. We performed pre-treatment of Vildagliptin (100 mg/kg) by subcutaneous injections for 3 days. Three weeks after tumor cell injection, the total lung was weighed and homogenized for quantification of GFP labeled tumor cells presented by fluorescence unit per body weight (FU/BW). Both, pre- and post-treatment of Vildagliptin significantly decreased the size of tumor in mice (**A**, **B**) without change of bodyweight within these 3 weeks (Suppl. 4). Gross anatomy of metastases developed by MC38 cell line injection is shown (**C**, *arrows* indicate tumor) 3 weeks after injection of the MC38 cell line (100×10^3 cells/g mouse) into the inferior vena cava (n = 5); *L* lung, *H* heart, *T* tumor. (**p = 0.0042, *p = 0.041)

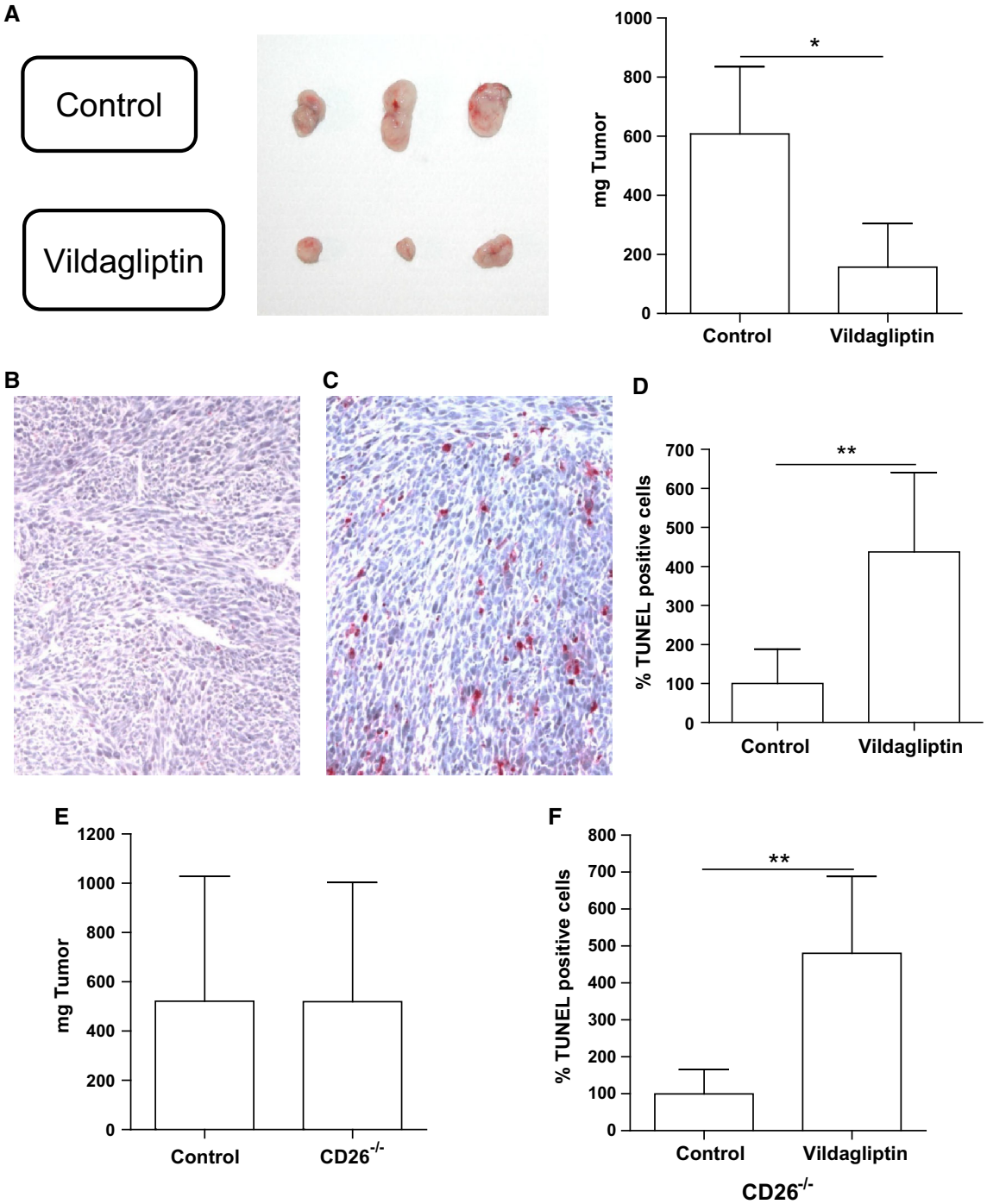

Fig. 4 Reduction of tumor size in the subcutaneously implanted MC38 tumor cell line by Vildagliptin treatment. MC38 cells (1×10^6 cells/mouse) were injected underneath the skin of syngeneic mice. Vildagliptin was administered in drinking water (~40 mg/kg/day). Two weeks after tumor cell injection, the subcutaneously developed tumor was excised to measure the wet weight. Macroscopic picture of tumors separated from mice and weight of tumors (**A**). Compared to control (**B**), Vildagliptin increased apoptotic cells (**C** and **D**) shown by TUNEL stain (n = 4). Tumor in CD26$^{-/-}$ mice showed no difference from control (**E**), however, the treatment of Vildagliptin on tumors developed in CD26$^{-/-}$ mice showed significantly more TUNEL positive cells (**F**) (n = 5 for each group) (*p = 0.0113, **p = 0.007)

grown in CD26$^{-/-}$ mice was not changed (Fig. 4E). However, the additional treatment of Vildagliptin on CD26$^{-/-}$KO mice increased the TUNEL positivity in these tumors, indicating that CD26-bearing cancer cells were indeed targeted in contrast to CD26$^{-/-}$ mice which do have to a much lesser degree CD26 activity (Fig. 4F).

Vildagliptin exerts its effect via decreased autophagy

Autophagy is recognized for its importance in maintaining tumor metabolism, resistance to chemotherapeutics, and survival under stress condition [25, 26]. Therefore, we analyzed markers that are related to autophagy in mouse tumor samples which developed upon MC38 cell line injection. The autophagy markers LC3, p62, and ATF4 were consistently decreased by Vildagliptin treatment within lung metastases and also in subcutaneously grown tumors. (Figure 5A, B; Supple. 3D–I). To test the effect of autophagy inhibition directly on the metabolic activity of cell lines in vitro, we challenged the cell lines by autophagy inhibitors in serum free condition for 3 days. Both autophagy inhibitors (3MA and SP600125) significantly decreased the metabolic activity of MC38 cell line (Fig. 5C). In parallel with the regulation of autophagy by Vildagliptin treatment, the levels of inhibitor of differentiation 1 (ID1) and its downstream target, metabolism determinant, AKT were downregulated by Vildagliptin treatment (Suppl. 5).

Vildagliptin downregulates cell cycle mediators in vitro and in vivo

In order to elucidate mechanisms through which the effect of Vildagliptin was exerted, we tested Vildagliptin on cell cycle mediators in non-cytotoxic concentrations. Vildagliptin (82 and 164 μM respectively) was incubated with the MC38 cell line. This treatment modulated the cell cycle-determining factor pCDC2 (Fig. 6A). Consistent with these in vitro results, phosphorylation of CDC2 was observed in subcutaneously induced tumors by Vildagliptin treatment (Fig. 6B). As a consequence of cell cycle

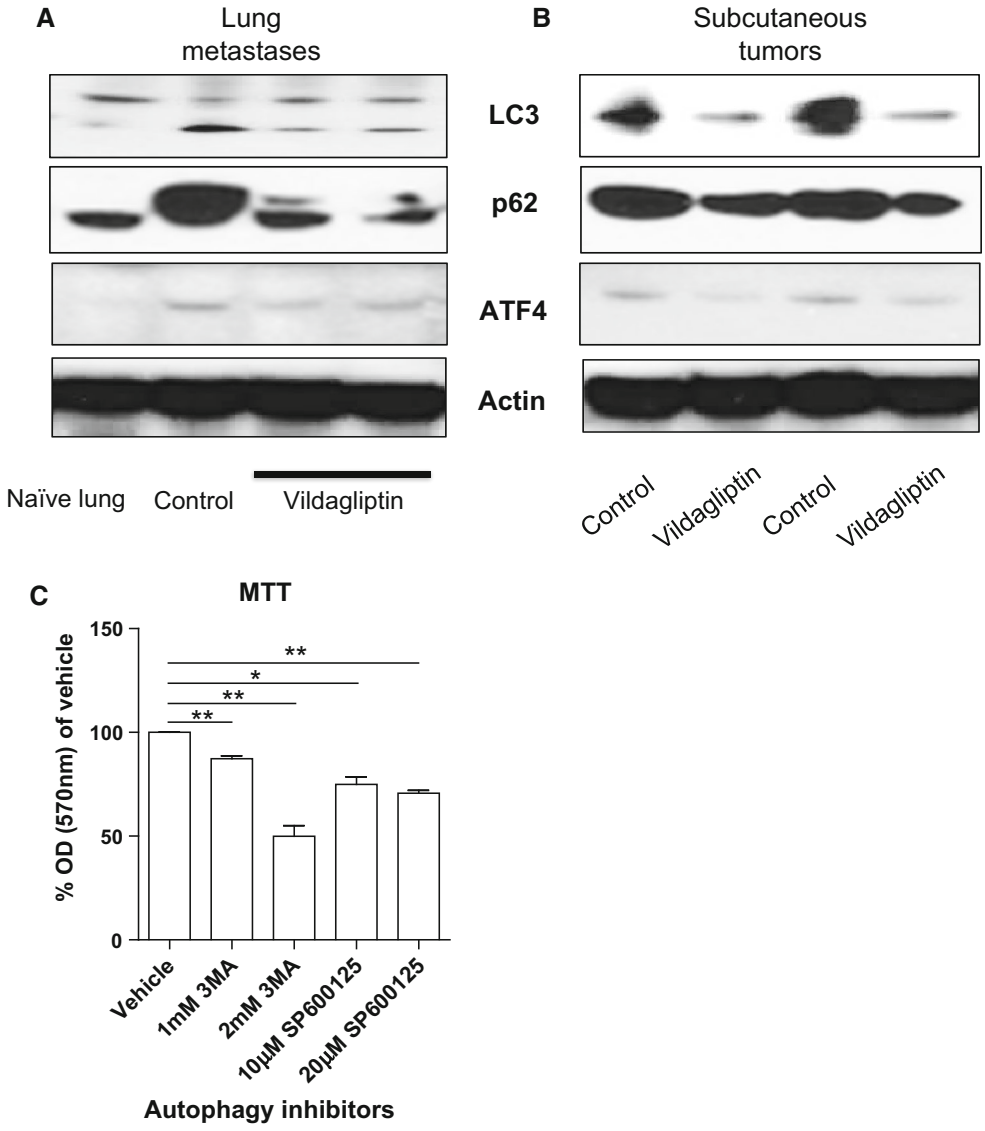

Fig. 5 Imbalance between autophagy and apoptosis by Vildagliptin treatment in tumor cells in vivo. The autophagy markers LC3, p62, and ATF4 were significantly decreased in tumors of lungs (**A**) and subcutaneous tumors (**B**) after MC38 cell line injection (100×10^3 cells/g mouse) (Suppl. 3D–I) ($n = 5$). The treatment by autophagy inhibitors (3MA, SP600125) on the MC38 cell line in vitro also showed a significant reduction of tumor cell metabolic activity after 3 days of incubation in serum free media (**C**)

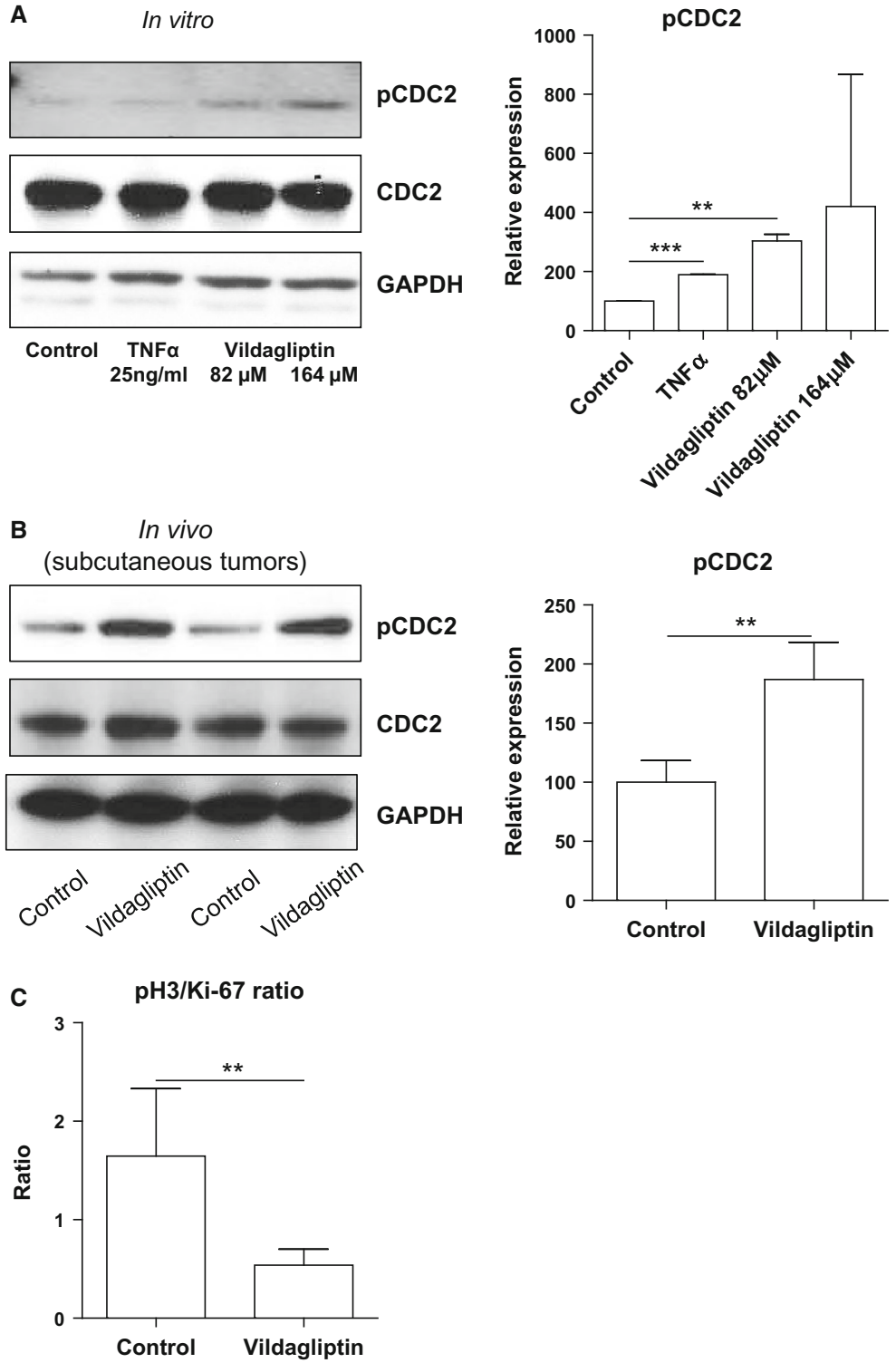

Fig. 6 Inhibition of the cell cycle by Vildagliptin treatment in vitro and in vivo. To show an effect of Vildagliptin on tumor cell growth, we added Vildagliptin to the MC38 cell line. After 8 h of Vildagliptin treatment, tumor cells were harvested for western blotting analysis of the G2/M phase driver pCDC2, CDC2, and GAPDH. By Vildagliptin treatment, CDC2 was inhibited by phosphorylation at the Tyr15 site (**A**). Consistent with the in vitro data, pCDC2 level was significantly elevated in subcutaneous tumor samples (**B**). Moreover, the ratio of pH3/Ki-67 as a mitosis marker was significantly decreased in Vildagliptin-treated tumor samples in vivo (**C**) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

interruption by Vildagliptin treatment, there was a significant modulation of the mitotic activity as reflected by a decreased pH3/Ki-67 ratio (Fig. 6C). Conclusively, cell cycle progression and proliferation in both, in vitro and in vivo, seems to be negatively regulated by Vildagliptin treatment.

Discussion

In this pre-clinical study, we found that a clinically established CD26/DPP4-inhibitor, namely Vildagliptin, has an anti-tumor effect against colorectal lung metastases in mice. Vildagliptin exerted its effects via a reduction of autophagy with the consequence of a decreased proliferation of cells and an increase of tumor apoptosis.

CD26/DPP4 have been described before to be involved in lung metastatic disease either in CD26/DPP4-deficient animal models or by using neutralizing CD26/DPP4 antibodies: when blocking CD26/DPP4 on lung endothelial cells, the interaction between lung-metastatic rat breast cancer cells and fibronectin was shown to be inhibited thus reducing at least in part the adhesion to endothelium and thereby the metastatic spread of cancer cells [27]. Alternatively, the development of lung metastases from breast adenocarcinoma cells have been described to be reduced, also via decreased adhesion of cancer cells in CD26/DPP4-deficient F344 rats [28]. Pang et al. identified a subpopulation of CD26-positive cells uniformly presenting in both primary and metastatic tumors in colorectal cancer patients suffering from liver metastases, showing that CD26-positive cancer cells were associated with enhanced invasiveness and chemoresistance [13]. Authors showed in CD26-positive cells that mediators of epithelial to mesenchymal transition (EMT) contribute to the invasive phenotype and metastatic capacity. These results show that if CD26/DPP4 is expressed on cells, this molecule can be targeted for metastatic disease therapy. In line with the findings of Pang et al., we could also observe a reduction of EMT markers, suggesting that the EMT status of MC38 cells were at least in part affected by Vildagliptin, consecutively diminishing the growth of metastases. However, in order to strengthen this hypothesis, the effect of Vildagliptin on the growth of metastases needs to be evaluated by more mechanistic studies unraveling the extra- or intra-cellular modulation of the metastatic machinery targeted.

With regard to the growth of existing metastases, we found that autophagy played a key role. Autophagy is an active cellular response to intra- or extra-cellular stress including stress to the endoplasmic reticulum, deprivation of nutrient, and reactive oxygen species. Autophagy in oncology includes multiple modes depending on the cancer type and its environment. On the one hand, the inhibition of autophagy has been shown to promote tumor development which has been tested in various malignancies such as lung cancer, hepatocellular cancer, and lymphoma [29, 30]. On the other hand, autophagy can prevent the development of tumor. Indeed, our data suggest that the CD26/DPP4-inhibitor Vildagliptin induces autophagy. In consequence, there was an increased apoptosis of tumor cells as reflected by increased TUNEL staining.

Another key mechanism during tumor development, at the same time as another consequence of autophagy is the reduced proliferation of tumor cells. In this context, phosphorylation (p) of CDC2 represents the state of arrest of cell cycle before the start of mitosis. [31] Together with increased pCDC2 levels, we found a decreased ratio of pH3/Ki67, both supporting the hypothesis that the inhibition of autophagy results in cell cycle arrest.

Even though Vildagliptin is commercially employed as a CD26/DPP4-inhibitor for the safe treatment of type II diabetes, it is also known to inhibit DPP8 and 9, and FAP. [32, 33] A recent study showed a synergistic effect of Vildagliptin on the anti-leukemic action of parthenolide that was completely mediated through its inhibition of DPP8/9 and not of CD26/DPP4 [34]. Although it is well possible that the effects of Vildagliptin shown in this study are not only mediated through CD26/DPP4, we could not detect any DPP8/9 activity in the metastases samples (Supple. 6).

With regard to the employment of Vildagliptin in routine clinical use for diabetic disease since years without showing relevant side effects (50–100 mg/person/day) and in the light of the data presented in this study, Vildagliptin seems to be a promising drug to also employ in metastatic disease. Yet, the concentrations given to mice here (40 mg/kg/day) were 100 times higher than applied in diabetic patients. However, when considering the activity of CD26/DPP4 and for an effective inhibition of CD26/DPP4 activity, exactly 100 times more Vildagliptin is necessary to inhibit the same activity in humans and mice. [35].

In conclusion, Vildagliptin decreased the growth of lung metastases by downregulating autophagy, increasing apoptosis, and arresting the cell cycle. On the base of these data, we suggest Vildagliptin for further clinical evaluation for the treatment of lung metastatic disease.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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