Am J Cancer Res 2015;5(6):2035-2046
www.ajcr.us /ISSN:2156-6976/ajcr0004925

Original Article

IFN-β is a potent inhibitor of insulin and insulin like growth factor stimulated proliferation and migration in human pancreatic cancer cells

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Received December 16, 2014; Accepted April 10, 2015; Epub May 15, 2015; Published June 1, 2015

Abstract: Introduction: Pancreatic cancer is a highly aggressive malignancy with few treatment options. The overexpression of several growth factors, including insulin and insulin-like growth factors (IGFs), can underlie the aggressive nature of this disease. Previous research has demonstrated potent effects of interferon (IFN)-β on pancreatic cancer cell growth, however up till now it is unknown whether IFN-β is able to counteract IGF1, IGF2 and insulin-induced pancreatic cancer cell proliferation and migration. Methods: Expression of IGF- and insulin receptors was determined and the stimulatory effects of IGF1, IGF2 and insulin on cell proliferation and migration, as well as the inhibitory effects of IFN-β were evaluated in 3 human pancreatic adenocarcinoma cell lines. Results: Both the insulin- and the IGF1 receptor were variably expressed in the cell lines. IGF1, IGF2 and insulin were capable of stimulating cell proliferation in all three cell lines, however cell migration was significantly enhanced only in the BxPC-3 cell line. IFN-β significantly inhibited IGF1-, IGF2- and insulin-stimulated proliferation in all three cell lines in a dose and time dependent manner. Furthermore, in the BxPC-3 cell line IFN-β significantly inhibited both basal and IGF1-, IGF2- and insulin-stimulated cell migration. Conclusion: Both IGF1, -2 and insulin were capable of stimulating proliferation and migration in human pancreatic cancer cells irrespective of the type of receptor expressed. This study demonstrates that insulin, in addition to IGF1 and IGF2, may play an important role in the progression of pancreatic cancer. Moreover, IFN-β strongly inhibits growth factor stimulated cell proliferation and migration. Our study supports previous findings which have suggested that IFN-β can be a potential promising anti-cancer agent in pancreatic cancer.

Keywords: Pancreatic cancer, interferon-beta, insulin, insulin-like growth factor, cell proliferation, cell migration

Introduction

Pancreatic cancer, with an overall 5-year survival of less than 6%, is the fourth leading cause of cancer related death in the western world [1]. At time of presentation over 80% of the patients are diagnosed with locally advanced or metastatic disease, indicating that pancreatic cancer can be considered as a systemic disease [2]. Several factors, including the overexpression of growth factors receptors, like insulin and insulin like growth factor (IGF) receptors, can underlie the highly aggressive nature of this disease [3, 4]. Additionally, previous research reported in 38-64% of the investigated pancreatic cancer specimens an overexpression of the IGF1 receptor (IGF1R), which was associated with more proliferating and invasive tumors leading to a poorer survival [5].

The IGF system is a highly complex system consisting of two growth factors (IGF1 and IGF2), two receptors (IGF1R and IGF2R) and six binding proteins (IGFBP 1-6) [6]. IGFs are very similar in function and structure to insulin, produced by the liver and several other tissues in response to pituitary growth hormone, and implicated as regulators of cell differentiation and cell proliferation in number of cell systems. Both IGF1 and -2 can interact with the IGF1R. However, only IGF2 can bind to the IGF2R, which is a scavenger receptor. Insulin signals via the insulin receptors (IRs) of which there are 2 known isoforms, the IR-A and IR-B. The IR-B receptor mainly activates the metabolic signaling pathway, whereas signaling via the IR-A induces primarily mitogenic effects. Besides that, insulin and IGFs may interact with each other's receptors, although with different affini-

ties [7, 8]. Furthermore, high levels of insulin can increase the hepatic IGF1 production by the upregulation of the growth hormone receptor (GHR). In addition, insulin may (independent from GH) directly increase IGF1 expression in the liver [9].

In cancer tissue in general, IGF1R is frequently overexpressed and in this respect pancreatic cancer is not different from other cancers [6, 10, 11]. Binding of IGFs to the IGF1 receptor induces activation of several pathways including the MAPK and PI3K pathways, which are associated with growth, proliferation, migration and the prevention of apoptosis [8]. Besides that, several epidemiological studies have also shown associations between diabetes mellitus and hyperinsulinemia on the one hand and an increased risk of cancer on the other hand [12-14]. Although the underlying mechanism still needs to be clarified, insulin and/or insulin receptors may play an important role in the development of cancer [15].

Although IGFs and their receptors have shown to be of importance in different cancer cell features it is, to the best of our knowledge, unknown to what extent IGFs (i.e. IGF1 and IGF2) are able of inducing cell migration in human pancreatic cancer cells. Furthermore, in many studies the role of insulin on tumor cell proliferation and migration is underexposed even though insulin is closely related to the IGF system.

Moreover, type I IFNs, particularly IFN-β, are able to modulate the IGF system in tumor cells by the suppression of endogenous production of IGF2 and by inhibiting the expression of the IGF1R [16]. Type I interferons (e.g. IFN-α and -β) are cytokines that are able to inhibit cell proliferation, induce apoptosis, block cell cycle and to sensitize tumor cells for chemo-and radiotherapy [17]. In a recent study, we have demonstrated these anti-tumor effects in human pancreatic cancer cells, in which the effects of IFN-β were significantly more potent compared to IFN-α [18]. However, it is unclear to what extent IFN-β is capable of inhibiting basal and growth factor stimulated cell proliferation and migration.

The aim of the present study is to evaluate the effect of IFN-β on the IGF1-, IGF2- and insulin-stimulated proliferation and migration of human pancreatic cancer cell lines.

Materials and methods

Cell lines and culture conditions

The human pancreatic cell lines BxPC-3, Hs 766T and PANC-1 were obtained from the American Type Culture Collection (Rockville, MD, USA). All cell lines were allelotyped and the DNA (STR) profile corresponded with the profile provided by the ATCC. The cells were cultured in a humidified incubator at 5% CO_2 and 37°C. The culture medium consisted of RPMI 1640 supplemented with 5% FCS, penicillin ($1x10^5$ U/L) and L-glutamine (2 mmol/l). Periodically, cells were confirmed as Mycoplasma-free. Cells were harvested with trypsine (0.05%) EDTA (0.53 mM) solution. Before plating, cells were counted microscopically using a standard hemocytometer. Trypan blue staining was used to determine cell viability. Media and supplements were obtained from GIBCO Bio-cult Europe (Invitrogen, Breda, The Netherlands).

Drugs and reagents

IGF1 and IGF2 were purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands). Human recombinant insulin was obtained from Novo Nordisk (Actrapid®, Bagsvaerd, Denmark). Human recombinant IFN-β-1a was acquired from Serono Inc. (Rebif, Rockland, MA). IGF1, IGF2 and IFN-β-1a were stored at -20°C, insulin was stored at 4°C. Stock solutions of IGF1 and IGF2 were constituted in 0.01 M of acetic acid, insulin and IFN-β were constituted in distilled water, all according to the manufacturer's instruction.

Quantitative RT-PCR

By quantitative RT-PCR mRNA expression of *IGF1, IGF2, IGF1R, IR-A, IR-B* and the housekeeping gene *hypoxanthine phosphoribosyltransferase (HPRT)*, was evaluated. The isolation of total RNA, complementary DNA (cDNA) synthesis and the primer and probe sequences (Sigma-Aldrich) that were used for the detection of *IGF1, IGF2, IGF1R, IR-A, IR-B* and *HPRT* have been described previously. [16, 19-21] Dilution curves were constructed for calculating the PCR efficiency for every primer set and have been described by van Adrichem *I* [21], Varewijck [20] and Vitale [16] After efficiency correction of target and reference gene transcripts (*HPRT*), the comparative threshold method, $2^{-\Delta Ct}$ was used to calculate the relative expression of genes.

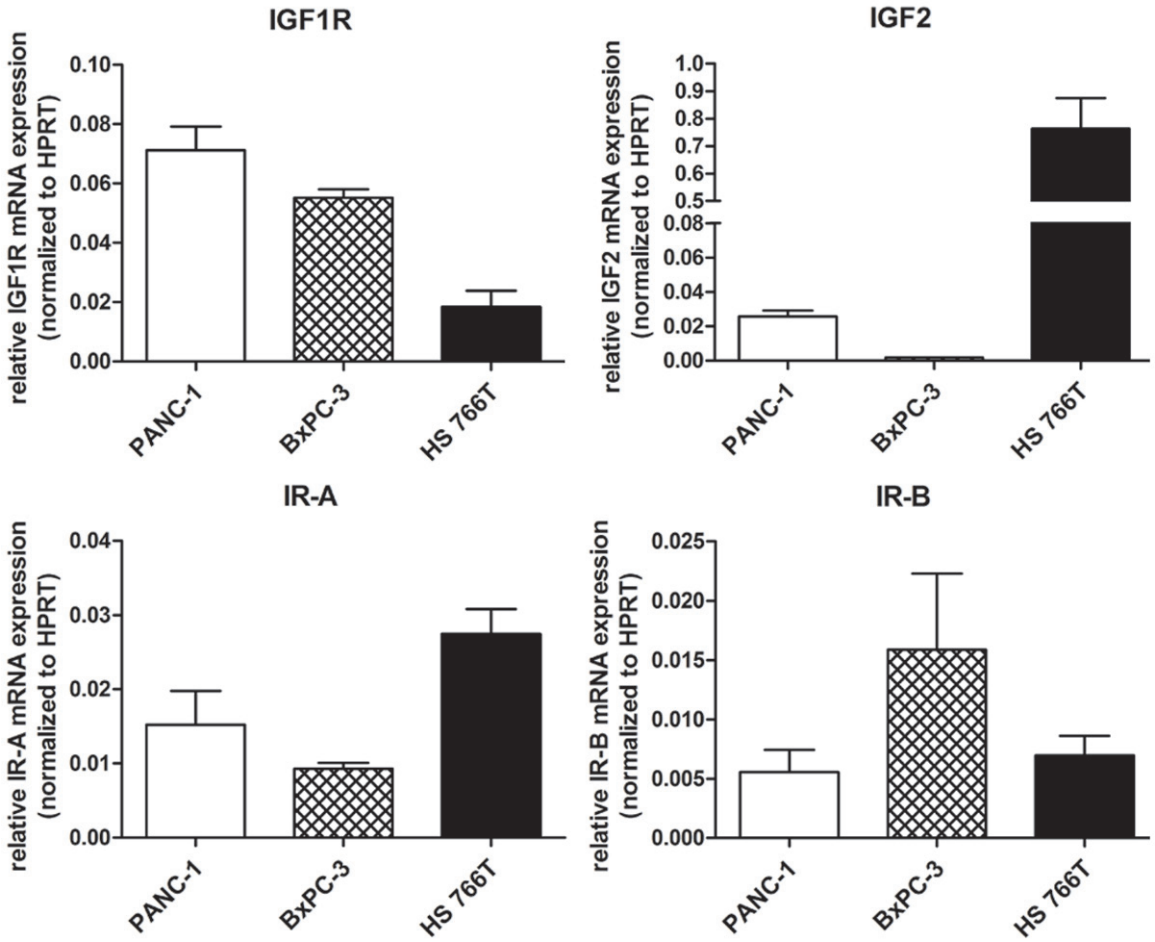

Figure 1. Relative expression of *IGF1R, IGF2, IGF-BP3, IRA* and *IRB* mRNA in 3 human pancreatic adenocarcinoma cell lines, normalized to *HPRT* mRNA. Values represent mean ± SEM.

Cell proliferation assay

For each cell line the optimal cell number plating density was determined (data not shown). After trypsinization, the cells were plated in 1 ml of medium in 24 well plates at the correct cell density. The plates were placed in a 37°C, 5% CO_2 incubator and cells were allowed to attach overnight. The next day, after washing the plates three times, the culture medium was replaced with 1 ml/well medium containing 0.5% FCS. Increasing concentrations ($1x10^{-10}$-10^{-8}) of IGF1, -II and insulin were added. Each treatment was performed in quadruplicate. After 3 and 7 days of treatment, the cells were harvested for DNA measurement. For the 7-day experiments, the medium containing 0.5% FCS was refreshed after 3 days and compounds were added again. As previously described, the measurement of total DNA contents was performed using the bisbenzimide fluorescent dye (Hoechst 33258) (Boehring Diagnostics, La Jolla, CA) [18].

Cell migration assay

The in vitro cell migration was measured by the scratch assay method described by Liang [22], with some modifications. After trypsinization, cells were plated in 2 ml of medium in poly-L-lysine coated 12-wells plates and placed in a 37°C, 5% CO_2 incubator. Cells were grown until a confluent monolayer was formed. With a 200ul pipet tip a scratch was made in the cell monolayer. The debris was removed by washing the cells once with 1 ml of growth medium and twice with 1 ml of medium containing 0.5% FCS. Hereafter, 2 ml of medium containing 0.5% FCS and the different compounds of interest were added. The ability of cells to migrate into the scratch area was assessed after 2, 4, 8 and 24 hours by comparing the 0- and 2,4,8

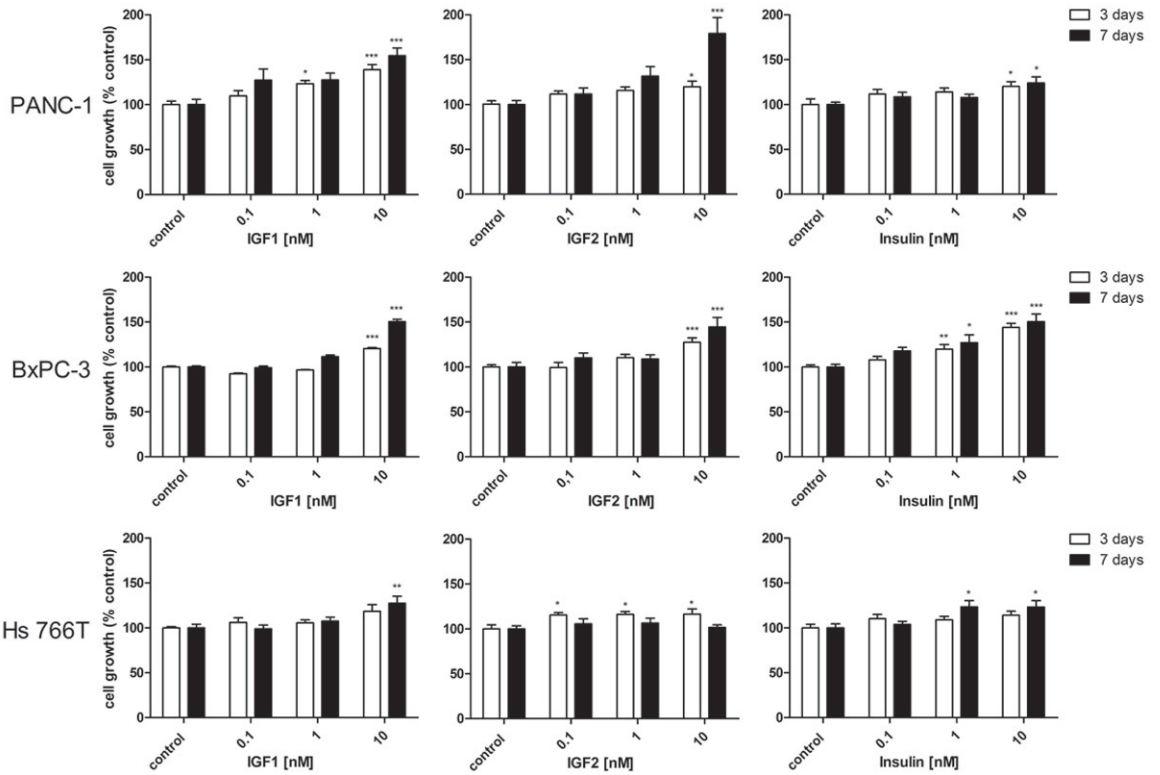

Figure 2. Treatment effects on cell proliferation after 3 days (open bars) and 7 days (black bars) of incubation with increasing concentrations of IGF1, IGF2 and insulin in 3 human pancreatic cancer cell lines. Values are expressed as the percentage of control and represent the mean ± SEM of at least 2 independent experiments in quadruplicate *p<0.05, **p<0.01 and ***p<0.001 versus control.

Table 1. Maximal stimulatory effect (Emax) by IGF1, IGF2 and insulin on proliferation of 3 human pancreatic adenocarcinoma cell lines after 3- (3 d) and 7-days (7 d) of incubation

		PANC-1		BxPC-3		HS 766T	
		Emax (%) 3 d	Emax (%) 7 d	Emax (%) 3 d	Emax (%) 7 d	Emax (%) 3 d	Emax (%) 7 d
IGF1	[10 nM]	39.1 ± 5.6***	54.5 ± 8.6***	20.4 ± 4.8***	50.4 ± 6.8***	18.4 ± 7.58	27.4 ± 7.9**
IGF2	[10 nM]	19.7 ± 6.3*	79.1 ± 17.8***	27.5 ± 4.9***	44.5 ± 10.5***	16.4 ± 5.8*	1.6 ± 2.9
INS	[10 nM]	20.2 ± 5.2*	24.0 ± 6.8*	44.0 ± 4.6***	50.4 ± 8.5***	14.2 ± 4.4	23.3 ± 6.9*

The maximal stimulatory effect is expressed as the percentage stimulation compared to the untreated control ± SEM. *p<0.05, **p<0.01 and ***p<0.001 versus control.

and 24-hour photomicrographs (Zeiss, Axiovert 40c, x50 magnification) of 4 fixed points along the scratch area. The percentage of non-recovered scratch area was calculated by dividing the non-recovered area after 2, 4, 8 and 24-hours by the initial scratch area (t=0) using the image software (http://rsb.info.nih.gov/ij/).

The scratch assay method was compared with a wound healing assay using the Cytoselect ™Wound Healing Assay Kit (Cell Biolabs, Inc., San Diego, USA). Briefly, after trypsinization cells were seeded in 1 ml of growth medium into 24-wells tissue culture plates containing inserts and incubated until a monolayer of cells was formed. After removing the inserts from the wells, the cells were treated according to the scratch assay method as described above.

Statistical analysis

All experiments were performed at least twice, with the exception of the migration assay, which was performed at least three times. For statistical analysis GraphPad Prism 5.0 (GraphPad software, San Diego, CA) was used. The com-

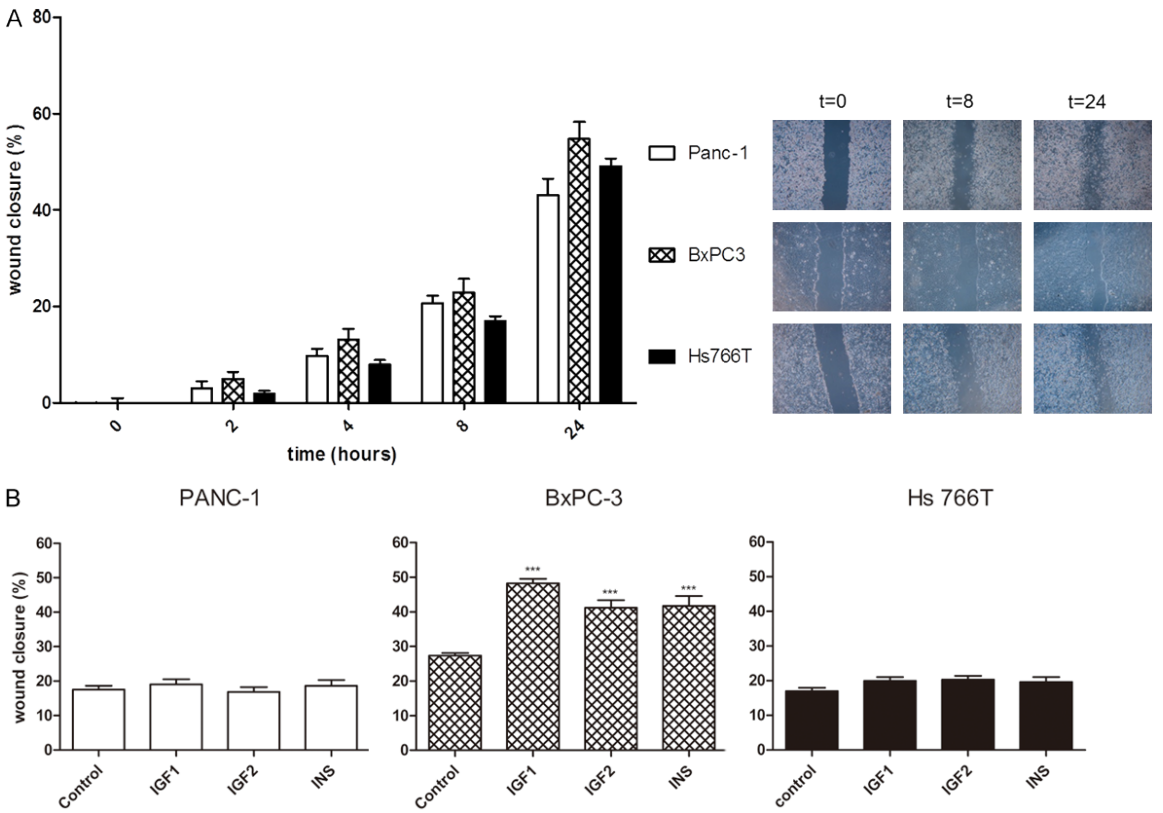

Figure 3. A. Left panel: Percentage cell migration in 3 human pancreatic cancer cell lines, after 2, 4, 8 and 24 hours after scratch. After scratch, medium was removed and cells were placed in medium containing only 0.5% FCS. The percentage of non-recovered wound area was calculated by dividing the non-recovered area after 2, 3, 8 and 24 hours by the initial wound area at 0 time; Right panel: Pictures of the scratch at 0 time and 8 and 24 hours after the scratch in 3 human pancreatic cancer cell lines. Original magnification x50; B. Percentage of cell migration after 8 hours of incubation with 10 nM IGF1, IGF2 or insulin in 3 human pancreatic cancer cell lines. Values are expressed as the percentage of wound closure compared to 0 time and represent the mean ± SEM of at least 3 independent experiments in triplicate, ***p<0.001 versus control.

parative statistical evaluation among groups was performed by a one-way ANOVA test. When significant differences were found, a comparison between groups was made using the Newman-Keuls test. In all analyses, values of $P<0.05$ were considered statistically significant. Data are reported as mean ± SEM.

Results

mRNA expression

By quantitative RT-PCR we analyzed the receptor mRNA expression of the *IGF1R, IGF1, IGF2*, *IR-A* and *IR-B* (**Figure 1**). All 3 human pancreatic adenocarcinoma cell lines expressed the *IGF1R* (highest in PANC-1), *IR-A* (highest in Hs 766T) and *IR-B* (highest in BxPC-3). None of the cell lines expressed detectable levels of *IGF1* mRNA (data not shown). *IGF2* mRNA was only expressed in the PANC-1 and Hs 766T cell lines, but not in the BxPC-3 cell line.

Growth factor stimulated proliferation

As shown in **Figure 2**, in two of the three cell lines growth factor stimulated proliferation was time- and dose dependent. After 3 days of incubation, at a concentration of 10 nM, IGF1 and insulin significantly stimulated the cell proliferation in 2 of the 3 cell lines. IGF2 significantly increased cell growth after 3 days in all three cell lines (**Figure 2**, open bars). After 7 days of incubation IGF1 and insulin stimulated the cell growth significantly at a concentration of 10 nM, in all three cell lines, whereas IGF2 increased cell proliferation in 2 of the 3 cell lines (**Figure 2**, black bars). **Table 1** shows maximal stimulatory effect of 3 and 7 days incubation with IGF1, IGF2 and insulin (all at 10 nM) in the 3 cell lines.

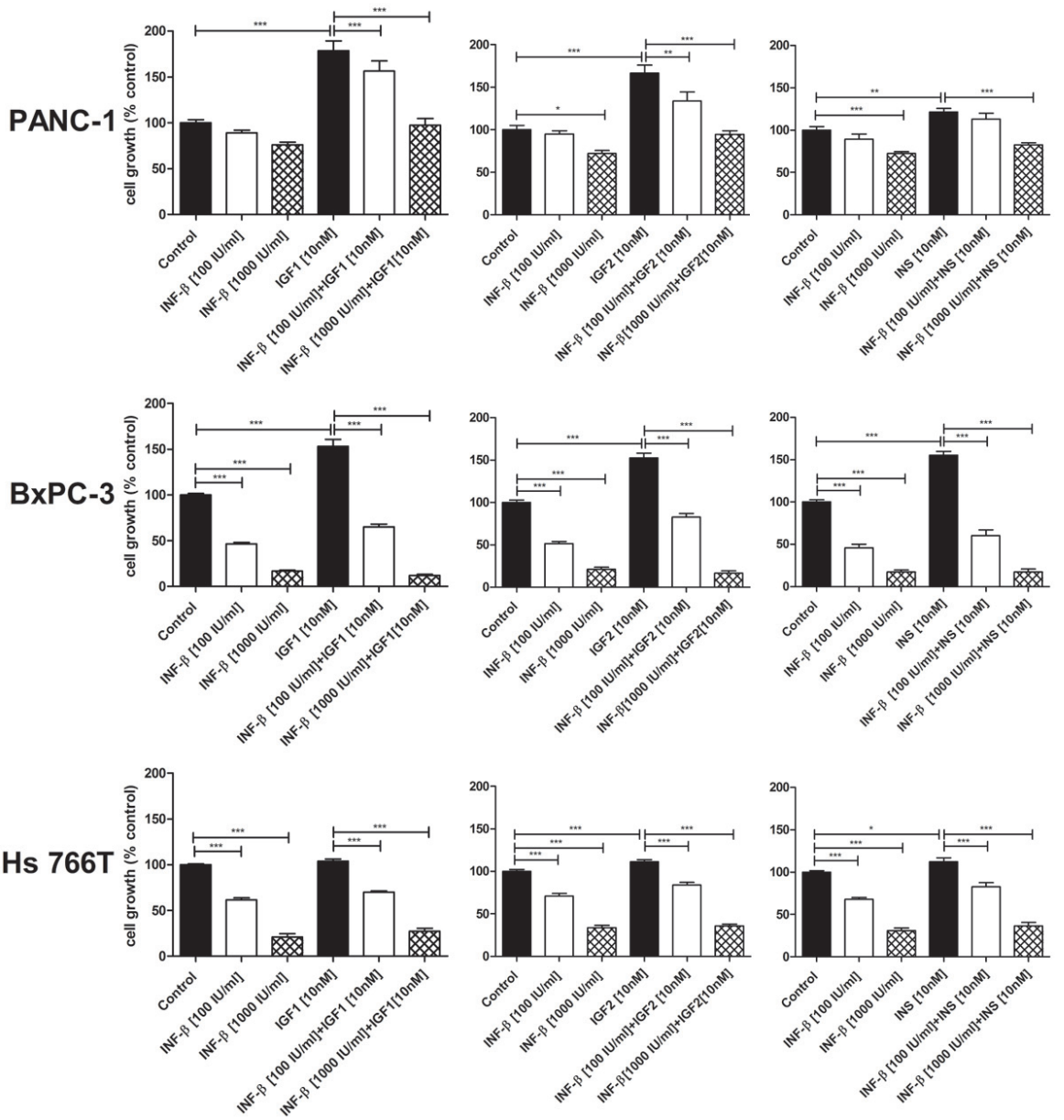

Figure 4. Effects of IFN-β treatment on cell proliferation after 7 days of incubation with and without the growth factors IGF1, IGF2 and insulin. The cells were incubated for 7 days with 100 IU/ml IFN-β, 1000 IU/ml IFN-β or 10 nM IGF1, IGF2 or insulin alone, or with the combination of 100 IU/ml or 1000 IU/ml IFN-β and 10 nM of IGF1, IGF2 or insulin. Values are expressed as the percentage of control and represent the mean ± SEM of at least 2 independent experiments in quadruplicate *p<0.05, **p<0.01 and ***p<0.001 versus control.

Basal and growth factor stimulated migration

To assess cell migration the scratch assay method was used in the three cell lines. First, basal cell migration was evaluated after 2, 4, 8 and 24 hours (**Figure 3A**). Although differences were relatively small, overall, the BxPC-3 cells migrated the fastest. After 8 hours 22.9% of the scratch was closed and after 24 hours closure was 54.7%. In the PANC-1 and the HS 766T cell line values were 20.7% and 16.9% after 8 hours, and 43.1% and 49% after 24 hours, respectively.

Furthermore, we evaluated the growth factor stimulated migration (**Figure 3B**). Of each compound (IGF1, IGF2 and insulin) a concentration of 10 nM was used and scratch closure was

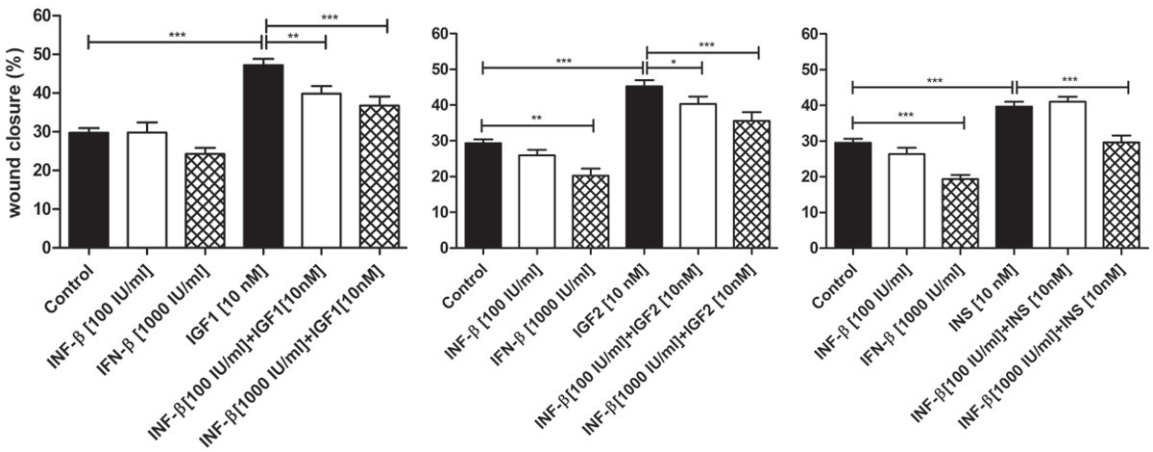

Figure 5. Percentage of cell migration after 8 hours of incubation with 100 IU/ml IFN-β, 1000 IU/ml IFN-β or 10 nM of IGF1, -2 or insulin alone, or with the combination of 100 IU/ml or 1000 IU/ml IFN-β and 10 nM of IGF-I, -II or insulin in the BxPC-3 cell line. Migration after 8 hours of incubation with 10 nM IGF1, -2 or insulin in 3 human pancreatic cancer cell lines. Values are expressed as the percentage of wound closure compared to 0 time and represent the mean ± SEM of at least 3 independent experiments in triplicate, *p<0.05, **p<0.01 and ***p<0.001 versus control.

assessed after 8 hours to avoid the possibility of involvement of cell growth. Only in the BxPC-3 cell line a statistically significant increase in migration by all growth factors was observed (p<0.001). After stimulation with IGF1, 8 hours after scratch 48.2% of the wound was closed (76.4% increase compared to control). After stimulation with IGF2 and insulin scratch closure was 41.2% and 41.7%, respectively (50.8% and 52.7% increase compared to control, respectively).

In addition, to validate the scratch assay, the Cytoselect ™Wound Healing Assay was used in the BxPC-3 cell line. No significant differences were observed between the two assays regarding basal and IGF1 stimulated cell migration (data not shown).

Inhibition of growth factor stimulated proliferation by IFN-β

We evaluated the effect of two different concentrations of IFN-β on the IGF1 (10 nM)-, IGF2 (10 nM)- and insulin (10 nM)-stimulated proliferation after 3 and 7 days of incubation. **Figure 4** illustrates the effects of 100 and 1000 IU/ml IFN-β, the maximal stimulatory effect of IGF1, II and insulin, as well as the combined effects of the compounds after 7 days of incubation. In all three cell lines, with the exception of the insulin-stimulated cell growth in the PANC-1 cell line, both 100 and 1000 IU/ml of IFN-β were capable of reducing the growth factor stimulated cell proliferation significantly. In addition to the dose-dependent effect of IFN-β there was also a time-dependent effect on basal proliferation as well as growth factor stimulated proliferation (data available on request).

Inhibition of growth factor stimulated migration by IFN-β

We also evaluated whether INF-β was able to inhibit growth factor stimulated migration. Given that only in the BxPC-3 cell line there was a significant increase of growth factor stimulated migration, the effect of IFN-β on growth factor induced migration was assessed only in this cell line. **Figure 5** shows the effects of 100 and 1000 IU/ml IFN-β on basal and growth factor stimulated cell migration after 8 hours of incubation. Similar to the experiments described in **Figure 3B**, at a concentration of 10 nM, all growth factors stimulated the cell migration in a statistically significant manner (p<0.001). Furthermore, there was a significant inhibition of basal cell migration by 1000 IU/ml IFN-β (p<0.001). Both 100 and 1000 IU/ml IFN-β were able to reduce IGF1 (p<0.01 and p<0.001, respectively) and IGF2 (p<0.05 and p<0.001, respectively) stimulated cell migration significantly. Regarding the insulin stimulated cell migration, only the higher concentration of 1000 IU/ml IFN-b was capable of reducing the cell migration significantly (p<0.01).

Discussion

Pancreatic cancer is a highly invasive malignancy with the potency to metastasize early. The highly aggressive nature of this disease can be explained by the overexpression of several factors, including insulin and insulin-like growth factors [3, 4]. Previous research has shown that the IGF1R is overexpressed in substantial part of pancreatic cancer specimens and associated with invasive and more proliferating tumors [5]. Besides that, it becomes more clearer that high levels of insulin can stimulate tumor growth. Moreover, several epidemiological studies associated diabetes mellitus, with the accompanying hyperinsulinism, and the use of insulin with an increased risk of certain cancers, as well as all-cause mortality [12-14, 23]. Additionally, given that insulin and IGFs may interact with each other's receptors, the role of insulin and the insulin receptor should not be neglected. Nevertheless, to the best of our knowledge, there are no studies yet comparing the effects of IGF1, IGF2 and insulin on both cell proliferation and migration of pancreatic cancer cells.

In the present study we first evaluated the mRNA expression of the growth factors and their receptors. Among the 3 human pancreatic adenocarcinoma cell lines, there was a considerable variability in expression of the IGF1R, IRA and IRB receptors. None of the cell lines expressed detectable levels of IGF1 mRNA and only the PANC-1 and Hs 766T cell lines expressed IGF2 mRNA.

Overall, the cell proliferative effects of IGF1, -2 and insulin were time- and dose dependent. However, in the Hs 766T cell line these effects compared to the other two cell lines, were much less pronounced, which might be explained by several reasons. First, in contrast to the other cell lines, IGF1R mRNA and protein expression is relatively low. Secondly, the IGF2 mRNA expression of this cell line is fairly high which can cause the IGF1 and insulin receptors to be already partially saturated. The addition of IGF1, IGF2 and insulin will, therefore, be minimally effective. Finally, the role of the family of IGF binding proteins (IGF-BPs) should not be ignored as they are capable of binding free circulating IGFs and thereby opposing the cell proliferating effects of IGFs. It has been described that in certain circumstances, like after serum starvation, IGF-BPs are also capable of stimulating cell growth [24]. Furthermore, IGF-BP3 and -5 are frequently overexpressed in pancreatic cancer and correlated with pancreatic cancer cell growth and a poorer survival. [4, 25-27] However, in the present study the role of IGFBP's was not evaluated.

In healthy subjects with normal insulin sensitivity, after food intake peripheral insulin concentrations rise to approximately 0.5 to 1.0 nM. It is estimated that peri-acinar concentrations of insulin are at least 20-fold higher than in the peripheral circulation, and should peak at 10-20 nM [23]. Although more than 95% of circulating IGFs are bound, in total circulating concentrations of IGFs are much higher than the concentrations of insulin. Generally, the normal IGF concentrations in adulthood can vary between 4 nM and 70 nM. However, this range strongly depends on factors like age, gender, diet and the used technique to determine the concentration. Besides that there is a large biological variation between individuals which makes it difficult to determine a generally applicable reference range. Therefore, given this wide spread in physiological concentrations, regarding the different growth factors, a fixed concentration of 10 nM was used to determine the IGF1, IGF2 and insulin stimulated migration, as well as the potential inhibitory effects of IFN-β on the growth factor stimulated proliferation and migration. However, at this concentration the growth factors are capable of binding each other receptors and therefore it is not possible to fully correlate the effects of the growth factors with their receptors. Nevertheless, this data does imply that, regardless of receptor type or level of expression, an IGF1-, IGF2- and/or Insulin-stimulated effect can be exerted. Besides that, cancer cells themselves are capable of producing growth factors, thereby creating an environment with elevated concentrations of the growth factors enabling themselves to proliferate and migrate [4, 25].

With the scratch assay method in vitro cell migration was evaluated. This method allows to study cell migration in vitro, which enables cell-cell interaction and to some extent mimics cell migration in vivo. Although the scratch assay method is a validated method to study cell migration we validated this method with the Cytoselect ™Wound Healing assay, since in the

scratch assay results can be compromised by the release of factors from damaged cells. However, we did not observe any differences between the two methods. As such, the scratch assay was a suitable method to study cell migration. Furthermore, by analyzing the growth factor stimulated migration 8 hours after scratch we excluded the possibility of the involvement of cell proliferation.

Only in the BxPC-3 cell line a significant increase in cell migration was observed after stimulation with the different growth factors. No significant increased cell migration was observed in the PANC-1 and Hs 766T cell line. Considering the relatively low IGF1R expression and the less pronounced effects of the growth factors on cell proliferation after 3 and 7 days in the Hs 766T cell line, the absence of stimulated cell migration after 8 hours could therefore be argued. Nevertheless, the absence of cell migration in the PANC-1 cell line is unexpected. It is known that cancer cell proliferation and migration are processes mediated by several molecules in different signaling cascades. AKT and its upstream regulator p13K are primarily involved in malignant cell proliferation, whereas ERK and the Ras/RAF/MAP kinase signaling pathways are more broadly involved in cellular functions like survival, proliferation, apoptosis and cell motility [3, 28, 29]. The epithelial-mesenchymal transition (EMT) is one of the initiators of the metastatic cascade and proteins involved in this EMT (e.g. Notch-2, Snail, N-Cadherin, Zeb, Vimentin and Slug) are also frequently overexpressed in pancreatic cancer [30-32]. As nicely investigated and described by Subramani [33] silencing the IGF1R resulted in an inhibition of proteins favoring pancreatic cancer EMT, additionally silencing of the receptor resulted as well in a downregulation of the active forms of AKT, P13K and mTOR by the upregulation of the tumor suppressor gene PTEN. Furthermore, by silencing of the receptor an effective inhibition of the active form of ERK was found. The authors concluded that the reduced capacity of the pancreatic cancer cells to proliferate and migrate was due to the suppression of key molecular pathways affected by the knockdown of the IGF1R. However, these effects became apparent after silencing the receptor for at least 48 hours. Additionally, the reduction in cell migration became statistically significant 72 hours after the scratch. Therefore, it can be hypothesized, that with respect to this cell line, 8 hours of incubation with the different growth factors may not have been sufficiently long enough to detect enhanced cancer cell migration.

As demonstrated in previous research, INF-β is a very potent molecule in inhibiting cell proliferation and in inducing apoptosis in pancreatic cancer cells [18, 34]. In addition, it is demonstrated that IFN-β is capable of modulating the IGF system by down regulating the expression of IGF1R and IGF2 mRNA in neuro-endocrine tumor cells [16]. However, despite these potent effects, there are no studies yet that evaluated the effects of IFN-β with respect to the IGF and insulin system in human pancreatic cancer cells. Therefore, in addition to the effects of insulin and insulin-like growth factors on pancreatic cancer cell proliferation and migration we investigated the inhibitory effects of IFN-β on the growth factor stimulated proliferation and migration as well. As shown in previous research, and reaffirmed in this study, IFN-β is a potent inhibitor of pancreatic cancer cell growth. Moreover, after 3 days of incubation with the different growth factors, 100 IU/ml of IFN-β was capable of inhibiting IGF1-, IGF2- and insulin-stimulated proliferation in two out of three cell lines, whereas a 1000 IU/ml of IFN-β was capable of inhibiting each growth factor stimulated proliferation in all three cell lines. These effects became even stronger after 7 days of incubation with both concentrations of IFN-β and the different growth factors. Besides the effect of IFN-β on growth factor stimulated proliferation we also studied the effect of IFN-β on growth factor stimulated migration. Since no significant effect of the growth factors was observed in the Hs 766T and PANC-1 cell line, we only evaluated the effect of IFN-β in the BxPC-3 cell line. In this cell line after 8 hours of incubation IFN-β (1000 IU/ml) significantly reduced basal migration as well as the migration stimulated by either IGF1, IGF2 or insulin. Additionally, 100 IU/ml of IFN-β inhibited both the IGF1, as well as the IGF2, stimulated migration significantly, which can be due to the fact that IFN-β is capable of down regulating the IGF1R [16]. Nevertheless, the inhibitory effects of IFN-β on cell migration are less pronounced as compared to the effects on cell proliferation, indicating that the associated signaling pathways are predominately involved in cell proliferation rather than in cell migration [35].

As clearly demonstrated in this study, insulin as well is capable of inducing cancer cell proliferation and migration. Besides that, it is known that IGFs and insulin can interact with each other receptors. Therefore, one can argue that targeting only the IGF1R will be insufficient in cancer treatment. Recently a new drug, OSI-906, has been developed that selectively inhibits autophosphorylation of both IGF1R and IR. OSI-906 hinders activation of downstream pathways and thereby inhibits migration, proliferation and survival in a variety of tumor cell lines [36-38]. Currently, OSI-906 is tested in advanced clinical studies. In future research, it would be interesting to test whether the anti-cancer effects of OSI-906 and IFN-β reinforce each other.

In conclusion, this study is the first that demonstrates the potent stimulatory effects of IGF1, -2 and insulin with respect to pancreatic cancer cell proliferation and migration. These results are in line with the highly aggressive nature of this disease and confirm the potential malignant potencies of insulin. In addition, we showed that IFN-β potently inhibits IGF1, IGF2 or insulin stimulated tumor cell growth, as well as the migration stimulated by these growth factors. In addition to the potent anti-tumor effects of IFN-β, these results therefore further favor the use of INF-β as part of the treatment options for patients with pancreatic cancer.

Disclosure of conflict of interest

None.

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