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Type I interferons as radiosensitisers for pancreatic cancer

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ABSTRACT

Background: Radiotherapy is an established treatment for malignant localised disease. Pancreatic cancer however seems relatively insensitive to this form of therapy.
Methods: Pancreatic cancer cell lines MiaPaca-2 and Panc-1 were pre-treated with 3000 IU/ml IFNα or 100 IU/ml IFNβ followed by 0, 2, 4, or 6 Gray (Gy) irradiation. Colony forming assay was used to assess the effects on cellgrowth. To measure the surviving fraction at the clinically relevant dose of 2 Gy (SF2), cells were pre-treated with 1000–10.000 IU/ml IFNα or 50–500 IU/ml IFNβ followed by 2 Gy irradiation.
Results: The plating efficiency was 49% for MiaPaca-2 and 22% for Panc-1. MiaPaca-2 was more radiosensitive than Panc-1 (surviving fraction of 0.28 versus 0.50 at 4 Gray). The SF2 of MiaPaca-2 was 0.77 while the SF2 of Panc-1 was 0.70. The SF2 significantly decreased after pretreatment with IFNα 1000 IU/ml ($p < 0.001$) and IFNβ 100 IU/ml ($p < 0.001$) in MiaPaca-2 and with IFNα 5000 IU/ml ($p < 0.001$) and IFNβ 100 IU/ml ($p < 0.01$) in Panc-1. The sensitising enhancement ratio (SER) for IFNα 3000 IU/ml was 2.15 in MiaPaca-2 and 1.90 in Panc-1. For IFNβ 100 IU/ml the SER was 1.72 for in MiaPaca-2 and 1.51 in Panc-1.
Conclusions: Type I interferons have radiosensitising effects in pancreatic cancer cell lines. This radiosensitising property might lead to an improved response to treatment in pancreatic cancer. Interferon β is the most promising drug due to its effect in clinically obtainable doses.

1. Introduction

Radiotherapy is an important treatment in cancer, especially for patients with advanced localised disease, with proven efficacy in many tumours.[1–5] Based on several randomised controlled trials studying the effect of adjuvant (chemo)radiotherapy, pancreatic and periampullary cancers are fairly radiotherapy resistant.[6–10]

Often chemotherapeutics (5-fluorouracil (5-FU), gemcitabine) are used as radiosensitisers. Besides their direct cytotoxic effects caused by incorporation of the drugs as modified nucleotides into the DNA, even low doses of these drugs can be effective in radiosensitisation. Interference with normal repair of radiation-induced DNA damage with an inappropriate progression through S phase is key in their radiosensitising properties causing late, unmanageable toxicities. A

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favourable side effect of their direct cytotoxicity is a relative increase in oxygenation, leading to an increased vulnerability for radiotherapy.[11,12] However, as mentioned, results of chemoradiotherapy are disappointing in pancreatic cancer.[6–10]

In several tumours radiosensitising properties of interferon alpha (IFNα) and interferon beta (IFNβ) have been demonstrated *in vitro*[13–17] as well as *in vivo*.[18,19] In pancreatic cancer cell lines, IFNα has already shown to act as radiosensitiser[20] and *in vivo* promising therapy results have been reported combining 5-FU, cisplatinum, and radiation therapy with IFNα alone (5-year survival rate of 55%) or followed by 2 cycles of gemcitabine (median survival 25 months) in patients with resected pancreatic adenocarcinoma.[21,22] These results are currently reinvestigated in the phase III CapRI study.[23]

Type I interferons such as IFNα and IFNβ, sort their effect through the same interferon receptor (IFNAR) with IFNβ having a higher affinity. *In vivo* studies showed that approximately 20% of pancreatic cancers express IFNARs and that expression of the interferon receptor correlates with a significant survival benefit in patients with resected pancreatic cancer.[24]

The exact mechanism by which type I interferons cause radiosensitisation is unclear. Possibly, concomitant treatment with IFNs causes an inappropriate progression of cells into S-phase, thereby interfering with repair of radiation-induced damage or increasing the proportion of lethal to sublethal damage.[17,25]

In this study we aim to gain insight in the radiosensitising abilities of type I interferons, especially IFNβ, in pancreatic cell lines. We decided to address these issues in colony forming assays, because besides the apoptotic effects of radiation and IFNs, the reproductive integrity of tumour cells (i.e. the capacity to produce an expanding colony of descendants, and therefore to regrow the tumour if left intact at the end of treatment) is of pivotal importance.

2. Materials and methods

2.1. Cell lines and culture conditions

The human pancreatic cell lines MiaPaCa-2 and Panc-1 were purchased from the American Type Culture Collection. The cells were cultured in a humidified incubator containing 5% CO_2 at 37°C. MiaPaca-2 was cultured in RPMI 1640 and Panc-1 in DMEM both supplemented with 10% FCS, penicillin (1×10^5 U/l), fungizone (0.5 mg/l) and L-glutamine (2 mmol/l). Periodically, the cells were tested for *Mycoplasma* contamination, which was not detected. Cells were harvested with trypsin (0.05%), EDTA (0.02%) and resuspended in medium. Before plating, the cells were counted microscopically using a standard haemocytometer. Tryphan Blue staining was used to assess cell viability, which always exceeded 95%. Media and supplements were obtained from GIBCO Bio-cult Europe (Invitrogen, Breda, The Netherlands).

2.2. Drugs and Reagents

Human recombinant IFN-α-2b (Intron-A) was obtained from Schering-Plough Corporation (Utrecht, The Netherlands), while human recombinant IFN-β-1a (Rebif) was acquired from Serono Benelux BV (Den Haag, The Netherlands). All compounds were stored at −20 °C, and the stock solution was constituted in distilled water according to the manufacturer instructions. Doses of 1000–10.000 IU/ml for IFNα and 50–500 IU/ml for IFNβ were used.

2.3. Irradiation

Cells were exposed to gamma radiation from a ^{137}Cs source at 70.9 cGy/min at room temperature under aerobic conditions. For radiation survival studies, cells were irradiated with 0, 2, 4, 6, 8, or 10 Gray. In the combined modality treatment, the IFN treatment was given before irradiation for 72 hours. Cells were irradiated with 0, 2, 4, or 6 Gray in the presence of the drug.

2.4. Colony forming assay

Cells were plated onto poly-L-lysine coated, 60-mm Petridishes (6–12 cells/cm^2) and cultured in complete medium for 2 weeks. Poly-L-lysine (10 μg/ml; Sigma–Aldrich, Zwijndrecht, The Netherlands) inhibited cells from dispersing from the growing colonies.

Dose response curves for IFNα, IFNβ and irradiation were established for both cell lines using a colony-forming assay. Therefore, seeded cells were allowed to attach for 24 hour prior to treatment with 1000–10.000 IU/ml IFNα, 50–500 IU/ml IFNβ or 0–10 Gray irradiation. Cell lines were treated with IFNs continuously and medium plus agents were replaced every three or four days. Fourteen days after seeding, colonies were fixed with 100% ethanol and stained with hematoxicilline to allow calculation of their average colony-forming efficiency. Colonies containing >50 cells were counted automatically with the MultiImage Light Cabinet from HpH Innitech Corporation.

Plating efficiency was defined as the mean number of colonies divided by the number of inoculated cells for control cultures not exposed to interferons or radiation. The surviving fraction (SF) was calculated as (mean number of colonies)/(number of inoculated cells × plating efficiency). The curve was plotted using X–Y log scatter (Graph Prism 3.0). Curve-fitting parameters α and β were determined.

2.5. Radiation enhancement by type I interferons

To asses radiation enhancement by type I interferons, cells were pretreated with IFNα 3000 IU/ml or IFNβ 100 IU/ml (doses resulting in approximately 50% decrease in surviving fraction in both cell lines) for 72 hours. Cell lines were irradiated with 0, 2, 4, or 6 Gray. Control plates without IFNs were irradiated simultaneously. Cell lines were treated with IFNs continuously and medium plus agents were replaced every three or four days. After 2 weeks, the formed colonies were fixed and stained to allow counting.

SF2 is the surviving fraction of cells that were irradiated at the clinically relevant dose of 2 Gray.

The sensitising enhancement ratio (SER) for interferon was calculated at the 37% survival level. The radiation dose that reduced the surviving colonies to 37% of the non-treated