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Differentiation of pancreatic carcinoma induced by retinoic acid or sodium butyrate: a morphological and molecular analysis of four cell lines

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Abstract The antiproliferative and differentiation-inducing effects of all-*trans* retinoic acid (RA) and sodium butyrate (SB) were investigated in four pancreatic ductal adenocarcinoma cell lines, two poorly differentiated ones (PT45 and PaTu-II), one moderately to poorly differentiated one (Panc-1) and one highly differentiated one (A818-1). Treatment with 20 μM RA resulted in moderate inhibition of cell growth in all cell lines, but clear evidence of cytodifferentiation (including elongated cell processes, increased rough endoplasmic reticulum, intensified immunostaining for the mucin marker M1) was found only in PT45 and Panc-1. These phenotypic changes were paralleled by upregulation of RAR (retinoic acid receptor)-α and -γ mRNA. SB (1 and 2 mM) treatment inhibited the cell growth of all cell lines much more prominently than RA. Cytodifferentiation was also largely restricted to PT45 and Panc-1. A noticeable phenomenon was enhancement of the expression of the neuroendocrine markers synaptophysin and Leu7 in Panc-1 cells. In conclusion, it is evident that the original differentiation status of cells and their responsiveness to the agents are not clearly associated, and that RA responsiveness correlates with upregulation of RAR-α and -γ mRNA.

Key words Pancreatic carcinoma · Growth · Differentiation · All-*trans* retinoic acid · Sodium butyrate

Dedicated to Prof. G. Seifert on the occasion of his 75th birthday

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Introduction

The grade of cellular differentiation is inversely correlated with increased proliferative capability in malignant cells, including those of pancreatic ductal carcinoma [33]. Investigation of the potential for inducing normal differentiation status in malignant cells can therefore help not only to improve our understanding of the mechanisms of tumorigenesis, but also to establish new strategies for cancer treatment. New concepts are particularly necessary in the treatment of pancreatic ductal adenocarcinoma. This tumor is one of the malignancies that have the most dismal prognosis. Despite great efforts to improve its early diagnosis, only about 10% of the patients have a resectable tumor at the time of diagnosis, and even in these patients the 5-year survival rate ranges below the 10% level in most recent series [46]. It is therefore worthwhile to investigate the potential for treating this tumor with differentiation inducers.

All-*trans* retinoic acid (RA), a naturally occurring analogue of vitamin A (retinol), and sodium butyrate (SB), a four-carbon fatty acid, are well-known and potent differentiation inducers in various cell types. Their ability to modulate phenotypic and molecular cell features has been studied in many tumors in vitro. Moreover, clinical studies have shown that oral treatment with RA can lead to transient remission in patients with acute promyelocytic leukemia [10, 11, 24, 45]. In other studies, 13-*cis* retinoic acid (a photoisomer of RA) has been shown to be effective in the chemoprevention of epithelial malignancies [22, 27, 32].

The cellular response to retinoids is thought to be mediated by two groups of nuclear receptors, retinoic acid receptors (RARs) [1, 7, 19, 29, 38, 49] and retinoid X receptors (RXRs) [20, 34]. Each retinoid receptor group consists of three major subtypes: α, β, and γ. RA is a li-

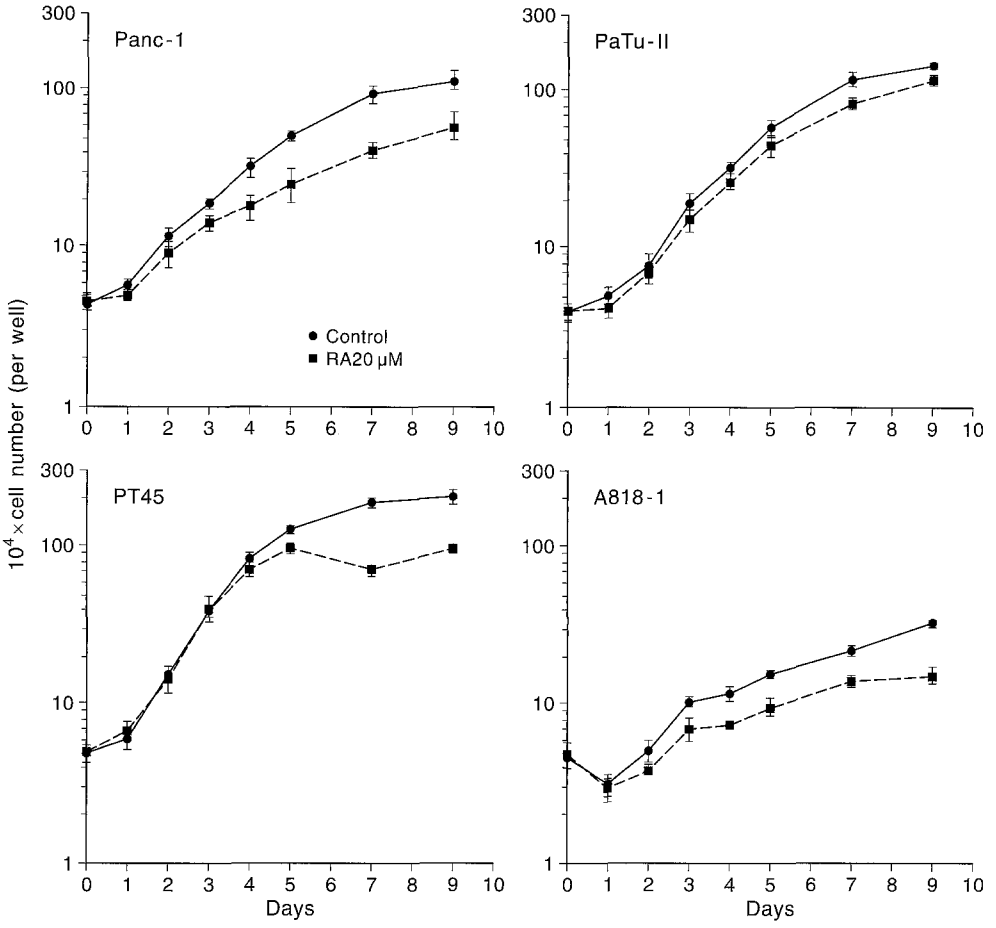

Fig. 1 Effect of 20 μM RA on the growth of four pancreatic carcinoma cell lines. Each *point* represents mean±SD of five tests

gand for RARs, while the ligand for RXRs is considered to be 9-*cis* retinoic acid [21, 31]. The RARs form heterodimers with RXRs and seem to bind directly to the promoter regions of responsive genes regulating the expression of genes related to differentiation [9, 26, 50]. Since the three subtypes of RAR exhibit distinct patterns of tissue-specific expression, they might mediate distinct biological activities.

SB is a naturally occurring four-carbon fatty acid whose molecular mechanism of action is still unclear. SB alters chromatin structure by inhibiting histone deacetylation and increases the nuclease sensitivity of chromatin [28], but no correlation between this increased sensitivity to nuclease and transcriptional activation in the rat hepatoma cell line H4IIE has been found [4]. Recent studies showed that a possible mechanism is the interaction of SB with cellular transcription factors [5].

The effects of RA on pancreatic cancer cells have been investigated in only a few studies [6, 17, 41]. One group reported that RA increased carbonic anhydrase II homologous RNA in the ductal pancreatic cancer cell line BxPC-3 [17], and another stressed the antiproliferative effect of RA not only on exocrine cancer cells (Panc-1 and MIA PaCa-2) but also on endocrine cancer cells (RIN-5mF and BON) [6]. Moreover, another study showed that RA treatment inhibited the growth of ductal cancer cells (Capan-1, Capan-2, Panc-1 and DAN-G) in a time- and dose-dependent manner and induced a more highly differentiated phenotype in these cells, as shown by morphological criteria and increased expression of carbonic anhydrase II [41].

The effects of SB have been examined in at least four cell lines. In Capan-1 cells, SB was found to induce increased alkaline phosphatase levels and CEA expression and to decrease transforming growth factor alpha expression and colony-forming efficiency [3, 15, 16, 35]. In Panc-1 and MIA PaCa2 cells, SB also decreased the colony-forming efficiency and increased the alkaline phosphatase activity [35]. In another study, SB caused an increase in cytoplasmic secretory elements in Panc-1 cells and made a poorly differentiated cell clone of the HPAF cell line indistinguishable from a more highly differentiated cell clone [37].

The aim of this study was to test the in vitro sensitivity of several pancreatic ductal adenocarcinomas to RA and correlate the RA-induced changes with RAR subtype expression patterns. In addition, we examined the effects of SB on the same cell lines and compared the findings with those obtained with RA. Finally, we tested the hypothesis that the effectiveness of RA or SB can be predicted from the original differentiation status of the tumor cell.

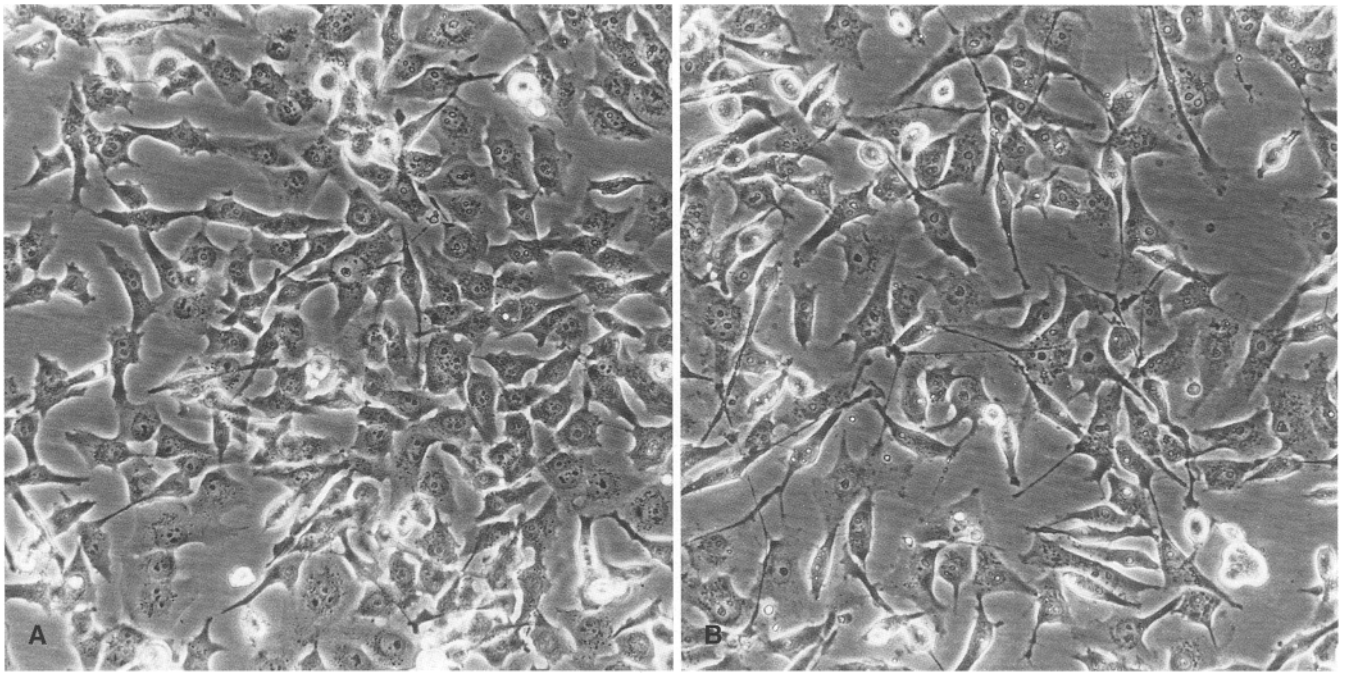

Fig. 2A, B Phase-contrast microscopy of PT45 cells on day 4. **A** Untreated cells; **B** cells treated with 20 μM RA. The treated cells appeared to be elongated and partly swollen. (×250)

Materials and methods

Cell lines

Four established human pancreatic ductal adenocarcinoma cell lines, PT45, PaTu-II, Panc-1 and A818-1, were used. Their grade of differentiation was determined according to a modification of Kern's ultrastructural classification of human pancreatic ductal adenocarcinoma [25]. The main criteria for determining cellular differentiation were uniformity of nuclei, development of microvilli and presence of cytoplasmic organelles. In brief, grade I is characterized by uniform nuclei, numerous microvilli, distinct desmosomes and many mucin granules; grade II, by uniform to irregular nuclei, few microvilli, few desmosomes and few mucin granules or the existence of small vesicles; and grade III, by irregular nuclei, abortive microvilli, no desmosomes, many small vesicles and no mucin granules. The PT45 cell line (gift from Dr. H. Kalthoff, Kiel, Germany) and the PaTu-II line (gift from Dr. M. v. Bulow, Mainz, Germany) are grade III, the Panc-1 cell line (gift from Dr. H. Kalthoff) is grade II–III and the A818-1 cell line (gift from Dr. H. Kalthoff) is grade I. These cell lines were maintained as monolayers in RPMI 1640 medium (Life Technologies, Paisely, UK), supplemented with 10% fetal calf serum, penicillin (50 IU/ml)/streptomycin (50 μg/ml) (Life Technologies), L-glutamine (2 mM) (Life Technologies) and sodium pyruvate (1 mM) (Sigma Chemicals, St. Louis, Mo.), and were grown in 5% CO_2/95% air at 37°C. These cultures were checked for mycoplasma contamination periodically.

Treatment with RA and SB

In order to determine the appropriate concentration of the agents, a preliminary experiment on cell growth was carried out by supplementing the culture medium with RA (Sigma Chemicals) in four different concentrations (5 μM, 10 μM, 20 μM and 33 μM) and with SB (Sigma Chemicals) in three different concentrations (1 mM, 2 mM and 5 mM). RA was dissolved in 100% ethanol and diluted with the growth medium to a final concentration of 0.1% ethanol for the experiments with 5, 10 and 20 μM RA or 0.17% ethanol for the experiment with 33 μM RA. The culture medium was changed on day 3 and then every other day. Both RA and SB caused a dose-dependent reduction in the cellular growth rate, which returned to the pretreatment rate after drug removal (data not shown). In this study, 20 μM of RA, and 1 and 2 mM of SB were chosen for the subsequent experiments because these concentrations of each agent induced prominent growth inhibition without significant cell death.

Cell growth

The cell growth curve was determined by plating the cells (5×10^4/well) in a 24-well microtiter plate. Five samples were taken for each time point. The cells were harvested at 1-day or 2-day intervals for 10 days, and the number of cells in each well was determined with a Neubauer cell counter.

Morphology

Serial observations were carried out under a phase contrast microscope. For transmission electron microscopy, cell pellets were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer. They were postfixed in 1% osmic acid and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate. The samples for the RA experiments were taken on days 4, 6 and 13, while the samples for the SB experiment were taken on days 1, 3 and 9.

Immunocytochemistry

Cell pellets were prepared at the same time points as for the electron-microscopic examination. They were fixed and dehydrated with toluene and ethanol and embedded in paraffin. The avidin-biotin peroxidase complex (Dako, Glostrup, Denmark) technique was used as the detection method. The monoclonal antibodies used were directed against the cytokeratins CK7 (1/100; Biogenex, San Ramon, USA), CK19 (1/10; gift from Dr. F. Raemaekers, Maastricht, The Netherlands), CK 8 and 18 (1/10; CAM5.2: Biogenesis, Bournemouth, UK), the mucin antigen M1

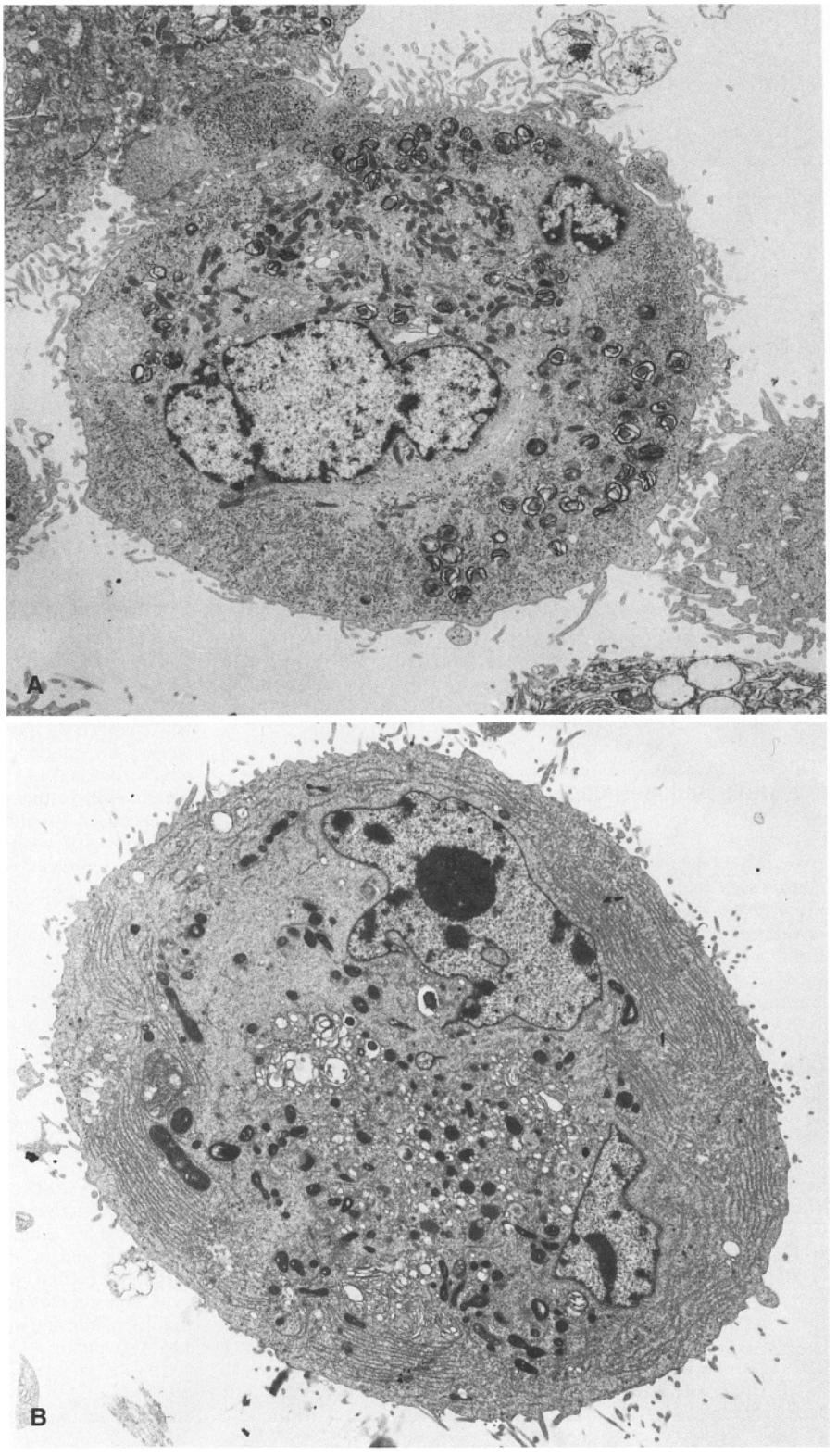

Fig. 3A, B Ultrastructure of Panc-1 cells on day 13. **A** Untreated tumor cell; **B** tumor cell treated with 20 μM RA. The treated cell shows a conspicuous development of rough endoplasmic reticulum. (×6,200)

(1/2,000; gift from Dr. E. Solcia, Pavia, Italy), CEA (1/20; BMA 130b, Behring, Marburg, Germany), B72.3 (1/20; Sorin Biomedica, Brussels, Belgium), CA19-9 (1/200; gift from Dr. H. Kalthoff, Kiel, Germany), vimentin (1/20; Boehringer, Mannheim, Germany), chromogranin A (1/1; Biogenex), synaptophysin (1/4,000; gift from Dr. B. Wiedenmann, Berlin, Germany), epidermal growth factor (1/30; Oncogene Science, Uniondale, N.Y.), epidermal growth factor receptor (1/30; Merck, Darmstadt, Germany), p53 (1/100; DO7, Novocastra, Newcastle, UK), intercellular adhesion molecule (1/500; Immunotech, Marseille, France) and neural cell adhesion molecule (1/500; Immunotech). Leu7 (1/3; Becton-Dickinson, Mountain View, Calif.) was used only if chromogranin A or synaptophysin immunostaining was positive. The immunocytochemical marker expression was evaluated on

two sections stained on different days. The staining intensity score was based on a summation of the grades given for intensity (negative 0, positive 1) and the percentage of positive cells (<5% 0; 5–20% 1; 21–50% 2; >50% 3). To check the specificity of the antibodies used, appropriate positive and negative control tissues were included.

Extraction of RNA and Northern blot analysis

Using the guanidine isothiocyanate-CsCl procedure [12], total RNA was extracted from the control cells and from exponentially growing cells treated with RA or SB at several time points. Total RNA (15 μg) was fractionated by electrophoresis on a 1% denaturing formaldehyde gel and transferred onto nylon membranes. The membranes (Hybond, Amersham, Bucks., UK) were hybridized to randomly primed ^{32}P probes derived from the following complementary DNA (cDNA) at high stringency: human RAR-α: cDNA probe ATTC [38]; human RAR-β: cDNA probe [7]; human RAR-γ: cDNA probe [29] (all 3 probes were gifts from Dr. P. Chambon). They were then autoradiographed to Hyperfilm MP (Amersham) at –80°C for several days. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a quantitative control [2]. Expression levels were determined by laser densitometry (UltroScan, LKB Pharmacia) and adjusted to the GAPDH signal.

Western blotting

The proteins from Panc-1 cells and rat brain tissue were extracted. Polyacrylamide gel electrophoresis was done in the presence of sodium dodecyl sulfate using 8% slab gels. The extracted cellular polypeptides were transferred onto nitrocellulose paper using a commercial transfer apparatus (Tranx-Blot, Bio-Rad, Munich, Germany) [44]. For protein staining of the nitrocellulose sheets, 0.2% Ponceau S (Sigma) was used. For blotting, the sheets were incubated overnight in 3% bovine serum albumin–phosphate-buffered saline solution. Then the sheets were exposed to rabbit antibodies to synaptophysin (dilution 1:500) for 60 min, washed, and exposed to peroxidase-coupled swine anti-rabbit antiserum (1:1,000, Dako). The immunodetection of proteins was done with the ECL detection system (Amersham) according to the manufacturer's instructions.

Results

RA-induced changes

The effect of 20 μM RA on cell growth varied from cell line to cell line (Fig. 1). Panc-1 appeared to be most sensitive and PaTu-II most resistant to RA. The growth-inhibitory effect of RA on PT45 cells appeared to be transient, since, despite continued treatment with RA, the cells resumed their basic growth rate from day 7 on.

The most prominent morphological changes in response to RA were seen with the phase-contrast microscope in the PT45 cell line, which showed elongated

Table 1 Immunocytochemical marker pattern[a] of pancreatic carcinoma cell lines before and after retinoic acid (*RA*) and sodium butyrate (*SB*) treatment (*CG-A* chromogranin A, *Syn* synaptophysin, *EGF* epidermal growth factor, *EGFR* epidermal growth factor receptor)

Cell line		CAM5.2	M1	CA19-9	CEA	CG-A	Syn	Leu7	p53	EGF	EGFR
Panc-1	Control	4	1	1	0	0	0	0	4	4	4
	RA 20 μM (day 13)	4	1	1	0	0	0	–	4	4	4
	SB 1 mM (day 9)	4	0	0	0	0	3	2	2	4	4
	SB 2 mM (day 9)	4	0	0	0	0	3	3	4	4	4
PT45	Control	4	0	0	0	0	0	–	3	3	4
	RA 20 μM (day 13)	4	2	0	0	0	0	–	4	3	4
	SB 1 mM (day 9)	4	0	0	0	0	0	–	3	4	4
	SB 2 mM (day 9)	4	0	0	0	0	0	–	3	4	4
PaTu-II	Control	4	0	4	2	0	0	–	4	3	4
	RA 20 μM (day 13)	4	0	4	3	0	0	–	4	3	4
	SB 1 mM (day 9)	4	0	3	1	0	0	–	4	4	3
	SB 2 mM (day 9)	4	0	3	1	0	0	–	4	4	4
A818-1	Control	4	2	4	2	0	0	–	0	4	4
	RA 20 μM (day 13)	4	2	4	2	0	0	–	0	4	4
	SB 1 mM (day 9)	4	3	4	3	0	0	–	0	4	4
	SB 2 mM (day 9)	4	3	4	4	0	0	–	0	4	4

[a] Staining intensity score was based on summation of the grades given for intensity (*0* negative, *1* positive) and percentage of positive cells (<5% 0, 5–20% 1, 20–50% 2, >50% 3)

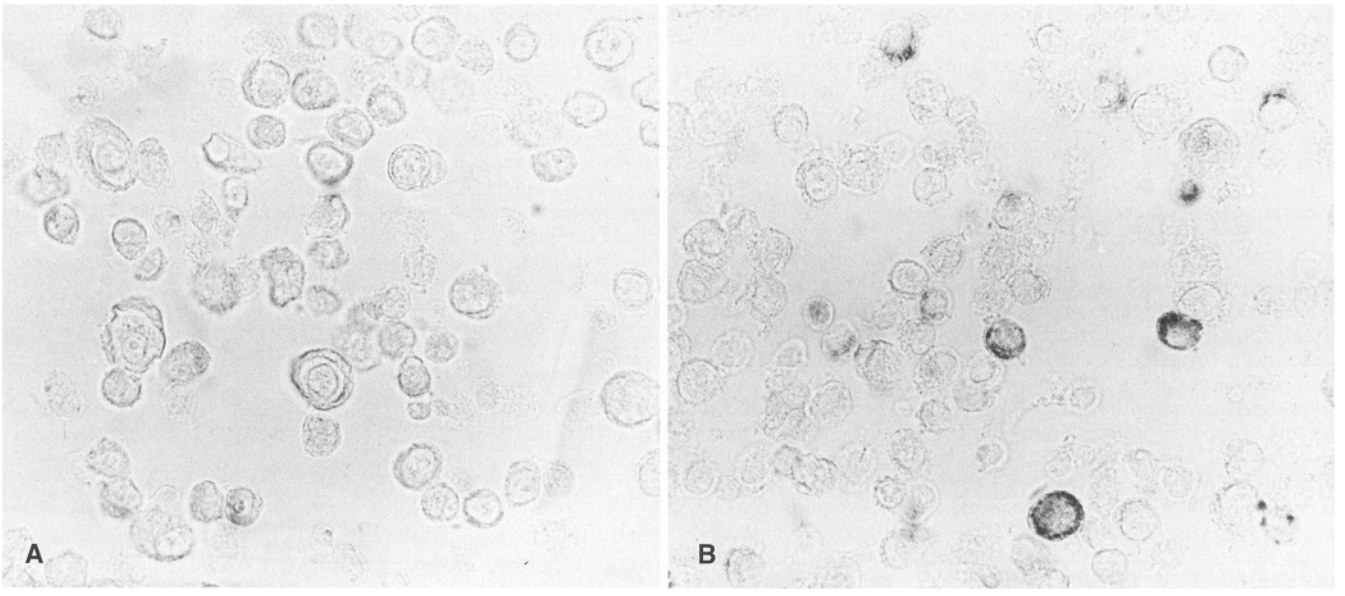

Fig. 4A, B Immunostaining of PT45 cells for the mucin marker M1 on day 13. **A** Untreated cells remain negative; **B** cells treated with 20 μM RA show M1 expression. (×600)

and partially enlarged cell processes (Fig. 2). These changes began to appear on day 3. Panc-1 cells underwent similar alterations but to a lesser extent. Minor alterations were seen in PaTu-II, with piling up of cells, and in A818-1 cells, with the appearance of cytoplasmic vacuoles. Ultrastructurally, PT45 and PaTu-II cells were graded as poorly differentiated, Panc-1 as moderately to poorly differentiated, and A818-1 as well differentiated [33]. In PT45 cells, RA caused an increase in cell size, which, however, was not associated with any significant development of cell organelles. In Panc-1 cells, there was an increased number of large cells on day 13, which displayed a distinctly increased rough endoplasmic reticulum (Fig. 3). In A818-1 cells, some cells with intracellular lumina had appeared during the treatment. PaTu-II cells did not change obviously during RA treatment.

Except for M1, RA treatment had no significant influence on the expression of the various markers tested in the four cell lines (see Table 1). M1, a mucin antigen known to occur in most pancreatic ductal adenocarcinomas [42], showed enhanced expression in PT45 cells (Fig. 4).

Two transcripts of RAR-α mRNA, 3.2 kb and 2.3 kb in size, were found in the four cell lines (Fig. 5). PT45 cells showed low baseline expression of both transcripts, which increased twofold after RA treatment for 7 days. Panc-1 also showed a twofold upregulation of the 2.3-kb transcript, which reached its peak after day 2. Both PaTu-II and A818-1 had high baseline levels, which were not altered by RA treatment. None of the cell lines tested expressed any detectable RAR-β mRNA either before or after RA treatment (data not shown). Prolonged exposure of the autoradiographs did not change these negative results. Two transcripts of RAR-γ mRNA, 3.5 kb and 3.3 kb in size, were observed in Panc-1 and PaTu-II, while PT45 and A818-1 contained only one 3.3 kb RAR-γ transcript. Basal expression of the 3.3-kb transcript was weak in both PT45 and Panc-1, but was upregulated fourfold and sixfold, respectively, on day 6 of RA treatment. In Panc-1 cells the 3.5-kb transcript was also increased threefold on day 6. Both PaTu-II and A818-1 had high RAR-γ baseline levels. However, they did not show significant changes after RA treatment.

SB induced changes

SB induced strong and early growth inhibition in all four cell lines (Fig. 6).

Phase-contrast microscopy revealed marked morphological changes in PT45 and Panc-1 cells. The changes in PT45 were similar to those seen after RA treatment but appeared earlier (days 1–3). Panc-1 cells became larger and flattened, showing filamentous protrusions. They were more marked with 2 mM than with 1 mM SB. PaTu-II cells only showed a tendency to become larger under SB treatment, while A818-1 cells remained unchanged. The ultrastructural changes in the PT45 and Panc-1 cell lines were characterized by an increase in cell size and in the number of cells with lipid vesicles and intracellular lumina. In addition, there was an increase in the number of cells with small smooth-surfaced vesicles, which seemed to be immature mucin granules in the PT45 cell line, and of cells with perinuclear microfilaments in the Panc-1 cell line. Neither A818-1 nor PaTu-II revealed significant alterations.

SB treatment did not significantly change the expression of the various markers tested in three of the four cell lines (see Table 1). In the Panc-1 cell line, there was an increased expression of synaptophysin (Fig. 7) and Leu 7.

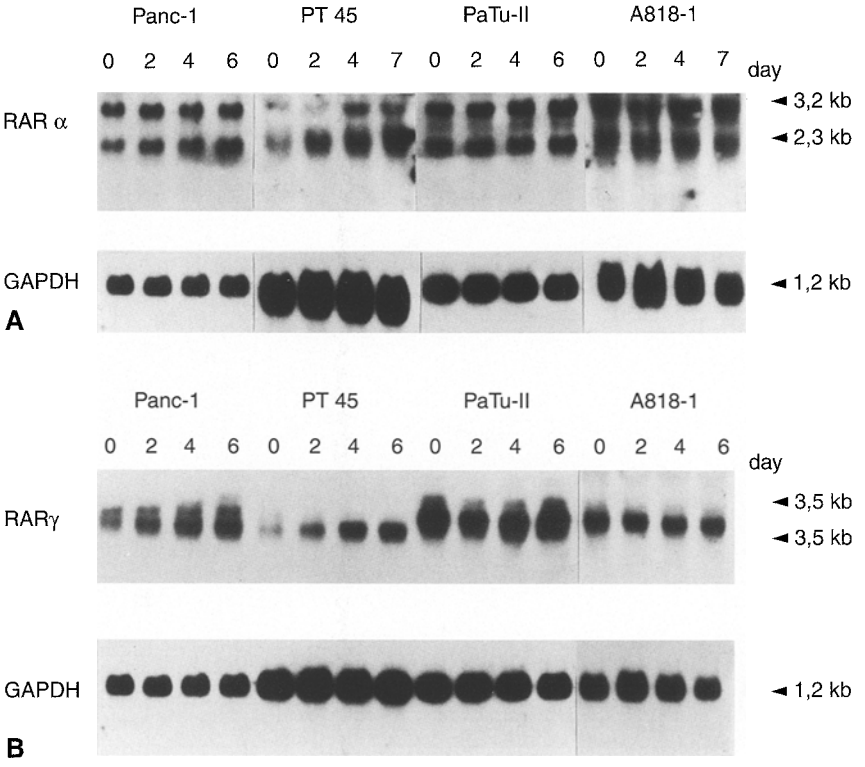

Fig. 5 Effect of RA on the expression of **A** RAR-α and **B** RAR-γ mRNAs in four pancreatic carcinoma cell lines on different days of treatment. GAPDH expression was used as control. Note the upregulation of RAR-α and RAR-γ in Panc-1 and PT45

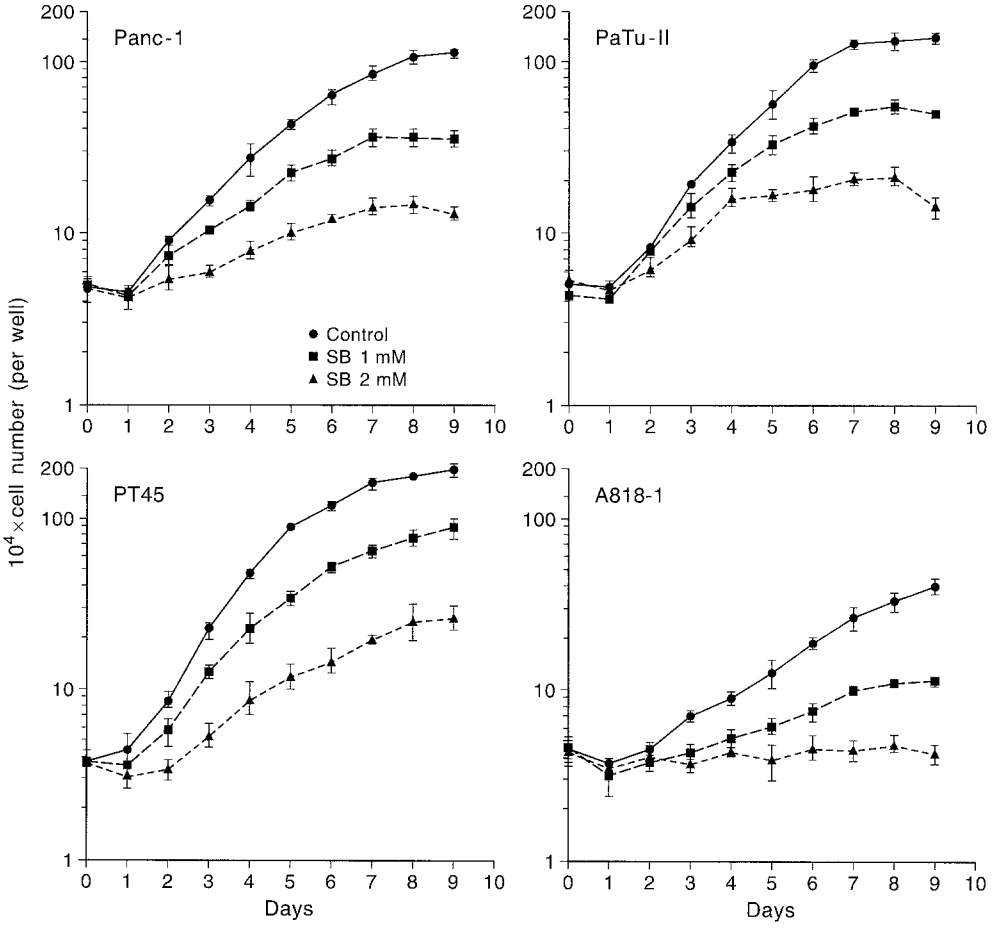

Fig. 6 Effect of SB on the growth of four pancreatic carcinoma cell lines. Each *point* represents mean±SD of five tests

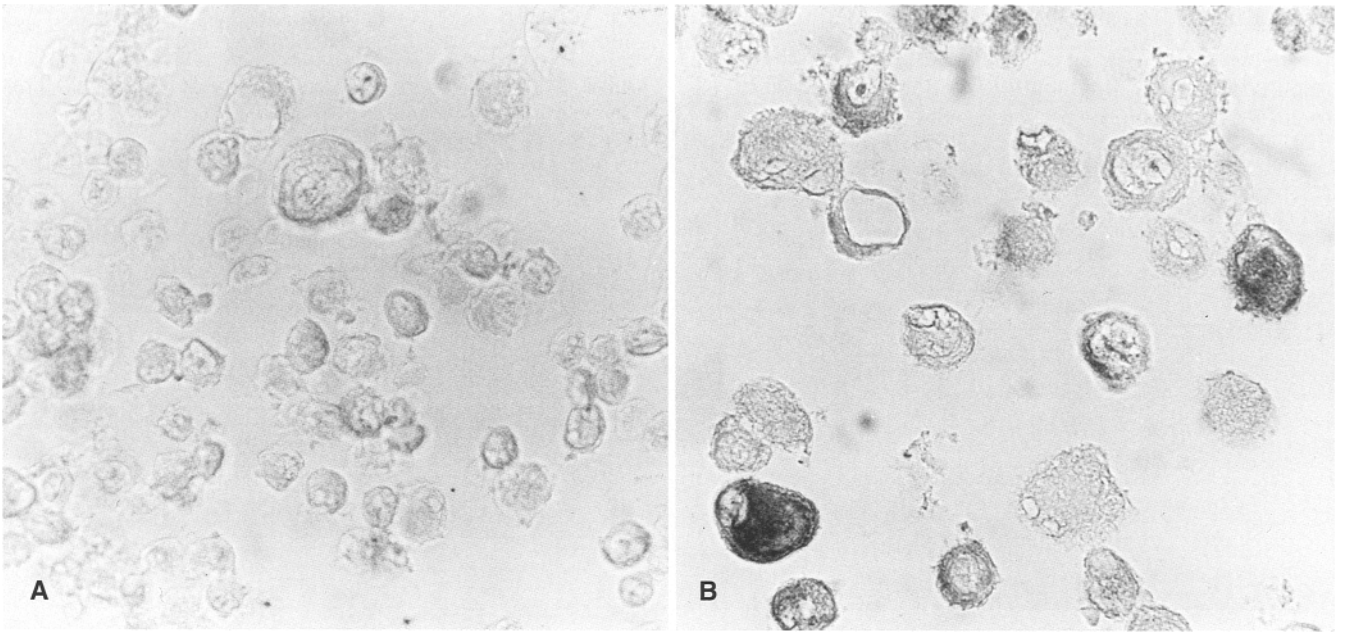

Fig. 7A, B Immunocytochemical staining for synaptophysin in Panc-1 cells on day 9. **A** Untreated cells; **B** cells treated with 2 mM SB. Synaptophysin is expressed by some cells. (×600)

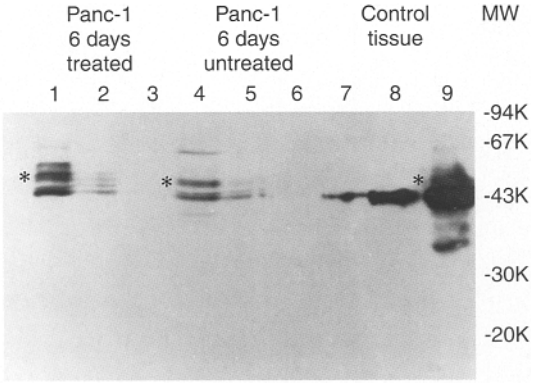

Fig. 8 Western blotting for the detection of synaptophysin protein in SB-treated and untreated Panc-1 cells. *Lanes 1, 2, 3* represent the protein extract from 450×10^3, 150×10^3 and 50×10^3 treated cells, respectively. *Lanes 4, 5, 6* represent the protein extract from 450×10^3, 150×10^3 and 50×10^3 untreated cells, respectively. *Lanes 7, 8, 9* represent the protein extract from 450×10^3, 150×10^3 and 50×10^3 cells, respectively, from control tissues (rat muscle, liver, brain). The proteins were detected by ECL (see Materials and methods). The positions of the protein markers (size in kDa) are indicated on the right. Distinct synaptophysin immunoreactivity can be found in *lanes 1, 4,* and *9* (see *asterisks*)

Synaptophysin was detected in both untreated and SB-treated Panc-1 cells (Fig. 8) by Western blotting.

Discussion

Our results show that among the four pancreatic carcinoma cell lines tested only two are clearly sensitive to the growth-inhibiting and differentiation-inducing effects of RA. Both sensitive cell lines (PT45 and Panc-1) are fast growing and poorly (or moderately to poorly) differentiated, while one of the unresponsive cell lines (A818-1) is well differentiated and slowly growing, so that sensitivity to RA might be thought to be associated with the cells' original differentiation status. However, this assumption is probably not true, because the fourth cell line (PaTu-II), which was also unresponsive to RA, was poorly differentiated. Hence the original differentiation status of the cell lines does not seem to predict their responsiveness to RA.

The concentration of RA found to be most effective in this study was 20 μM. In another recent investigation on pancreatic carcinoma cell lines a concentration of 10 μM RA was used [41]. Both RA concentrations are rather high compared with that used in investigations conducted on tumors other than pancreatic carcinomas [8, 13, 30, 43]. Pancreatic carcinomas may therefore be less sensitive to RA than other malignancies, notably in hematological disorders. However, it has recently been reported that even 0.1 μM RA led to significant growth inhibition of pancreatic ductal carcinoma cell lines [6]. Although it is difficult to explain this discrepancy, it may be partially attributed to the differences in the medium, including the vehicle for RA (ethanol vs dimethyl sulfoxide) and the concentration of fetal calf serum (10% vs 0.1–1%).

The phenotypic changes found in the two sensitive cell lines, PT45 and Panc-1, consisted in an elongation of cell processes and increase in rough endoplasmic lamellae and expression of the mucin marker M1. The molecular mechanisms that induce and regulate these changes are poorly understood, but it is thought that the action of RA at the gene level is mediated via nuclear receptors, RARs. RAR expression patterns induced by RA seem to be specific to each tumor cell line. For example, in some tumor cell systems, such as the human neuroblastoma cell line LA1-15n [13], the murine embryonal

carcinoma cell line F9 [48] and the murine melanoma cell line S91 [39], which are all known for their high sensitivity to RA, the phenotypic changes induced by RA are associated with a substantial upregulation of RAR-β, which is much more prominent than that of RAR-α and -γ. However, RAR-α appears to mediate the RA-induced growth inhibition of MCF-7 human breast cancer cells [14]. Moreover, RAR-γ plays an important role in the RA-mediated differentiation of the human teratocarcinoma cell line NETRA-2 clone D1 [36].

Our analysis of the mRNA levels of the three RAR subtypes α, β, and γ revealed a heterogeneous expression pattern in the RA-treated and untreated cells. Transcripts were only found for RAR-α and -γ mRNA, but not for RAR-β. This result closely corresponds to the data from a study by Rosewicz et al. combining Northern blotting with reverse transcriptase polymerase chain reaction analysis, which also showed very low expression of RAR-β [41]. From these basal expression patterns of RAR, these authors hypothesized that RAR-γ probably has a key role in retinoid action on human pancreatic ductal tumor cell lines [41]. However, they did not see any change in RAR expression during RA-induced tumor cell differentiation. Our data revealed significant RA-induced upregulation of RAR-α and -γ mRNA in the two cell lines (PT45 and Panc-1) with the most obvious phenotypic changes, although both cell lines had low baseline RAR-α and -γ mRNA expression levels. In the other two cell lines, A818-1 and PaTu-II, which were almost unresponsive to RA, baseline levels of RAR-α and -γ mRNA were high, but not markedly altered by RA treatment. These data may suggest that the effect of RA is mediated via RAR-α and -γ. As to the reason why the basal expression of RAR-α and -γ mRNA failed to reflect the cells' responsiveness to RA, we surmise that in the RA unresponsive cell lines the gene mutations underlying malignancy also involve the RA signal transduction system.

It is striking that neither our four cell lines nor those studied by Rosewicz et al. [41] were found to express demonstrable levels of RAR-β mRNA before, during or after RA treatment when examined with the Northern blot technique. Similar results have been obtained in lung and oral carcinoma cell lines [18, 23]. So far the significance of this finding is unclear.

SB also had a pronounced effect on growth and differentiation in the pancreatic carcinoma cell lines. Strongly dose-dependent growth reduction was noted by day 2 or day 3 and affected all four cell lines. However, like RA, SB particularly altered the morphology of PT45 and Panc-1 cells, causing clear changes in cell shape, organelle density and marker expression. A noticeable finding was the induction of neuroendocrine marker expression in the Panc-1 cell line. This was demonstrated by a positive immunoreaction for synaptophysin and Leu7. The negative reaction for chromogranin A (a protein occurring almost exclusively in neuroendocrine granules [47]) corresponded to the absence of neuroendocrine secretory granules at the ultrastructural level. Western blotting confirmed the presence of synaptophysin in Panc-1 cells and also showed that synaptophysin is already demonstrable in untreated cells, which were found by immunocytochemistry to be synaptophysin negative. This suggests that low-level synaptophysin expression is a feature of Panc-1 cells. These cells may therefore have a multidirectional differentiation potential which, in addition to exocrine features, also possesses an endocrine differentiation that may be enhanced by SB. Dual differentiation, combining exocrine and neuroendocrine features, has also recently been reported in the pancreatic cell line AR42J, which derives from a rat pancreatic acinar carcinoma [40].

In conclusion, our study demonstrates that the original differentiation status of pancreatic carcinoma cell lines does not predict the cells' responsiveness to RA and SB. The molecular mechanisms determining the responsiveness to these compounds do not therefore seem to be related to morphological features. Moreover, both substances exerted their strongest effects on the same cell lines, which suggests that both substances operate by way of related cellular mechanisms.

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