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ORIGINAL ARTICLE

Two peptides derived from ras-p21 induce either phenotypic reversion or tumor cell necrosis of ras-transformed human cancer cells

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Abstract
Purpose We investigated the effects of two peptides from the ras-p21 protein, corresponding to residues 35–47 (PNC-7) and 96–110 (PNC-2), on two ras-transformed human cancer cell lines, HT1080 fibrosarcoma and MIAPaCa-2 pancreatic cancer cell lines. In prior studies, we found that both peptides block oncogenic, but not insulin-activated wild-type, ras-p21-induced oocyte maturation. When linked to a transporter penetratin peptide, these peptides induce reversion of ras-transformed rat pancreatic cancer cells (TUC-3) to the untransformed phenotype.
Methods These peptides and a control peptide, linked to a penetratin peptide, were incubated with each cell lines. Cell counts were obtained over several weeks. The cause of cell death was determined by measuring caspase as an indicator of apoptosis and lactate dehydrogenase (LDH) as marker of necrosis. Since both peptides block the phosphorylation of jun-N-terminal kinase (JNK) in oocytes, we blotted cell lysates of the two cancer cell lines for the levels of phosphorylated JNK to determine if the peptides reduced these levels.
Results We find that both peptides, but not control peptides linked to the penetratin sequence, induce phenotypic reversion of the HT-1080 cell line but cause tumor cell necrosis of the MIA-PaCa-2 cell line. On the other hand, neither peptide has any effect on the viability of an untransformed pancreatic acinar cell line, BMRPA1. We find that, while total JNK levels remain constant during peptide treatment, phosphorylated JNK levels decrease dramatically, consistent with the mechanisms of action of these peptides.
Conclusion We conclude that these peptides block tumor but not normal cell growth likely by blocking oncogenic ras-p21-induced phosphorylation of JNK, an essential step on the oncogenic ras-p21-protein pathway. These peptides are therefore promising as possible anti-tumor agents.

Keywords Ras-p21 protein · Cell transformation · Ras peptides · Cancer cell lines · Phenotypic reversion · Tumor cell necrosis

Abbreviations

MAPK	Mitogen-activated protein kinase also called ERK

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JNK	Jun-N-terminal kinase
TOPK	Lymphokine-activated killer T-cell-originated protein kinase (TOPK)
DYRK1A	Dual-specificity-tyrosine-phosphorylation-regulated kinase-1A
Penetratin or leader sequence	Transmembrane-penetrating peptide
PNC-2	Ha-ras peptide, 96–110
PNC-7	Ha-ras peptide, 35–47
PNC-29	Negative control peptide from cytochrome P-450 (called X13) attached to penetratin sequence
CD-45-leader	Negative control peptide from the CD-45 antigen linked to penetratin sequence
LDH	Lactate dehydrogenase

Introduction

The ras-gene-encoded p21 protein becomes oncogenic when arbitrary single amino acids are substituted for the normally occurring ones, such as Gly 12 and Gln 61, in the wild type protein [1–3]. From the results of molecular modeling studies of the average conformations of the wild-type and multiple oncogenic forms of ras-p21, we have found that oncogenic amino acid substitutions induce stereotypical changes in the three-dimensional structures of specific domains of this protein [2, 3]. Two of these regions involve residues 35–47 of the switch 1 domain and residues 96–110. The former residues occur in a domain that has been implicated in the interaction of ras-p21 with a number of target proteins including GTPase activating protein (GAP), raf, the guanine nucleotide exchange-promoting protein, SOS and phosphoinositol-3-hydroxy kinase (PI3K) [2, 3]. The latter residues have been implicated in the interaction of ras-p21 with SOS [4, 5] and jun kinase (JNK) proteins [2, 3, 6, 7].

We have synthesized both peptides, called PNC-7 (residues 35–47) and PNC-2 (residues 96–110), and assayed them for their abilities to block oncogenic ras-p21 induction of maturation of *Xenopus laevis* oocytes [2, 3]. Injection of oncogenic but not wild-type ras-p21 into these oocytes induces oocyte maturation, i.e., completion of the second meiotic division, as measured by germinal vesicle membrane breakdown (GVBD) [8]. Insulin also induces oocyte maturation in a manner that requires activation of endogenous wild-type ras-p21 [9].

We have found that both PNC-7 and PNC-2 peptides block oncogenic (Val 12-containing) ras-p21-induced oocyte maturation but have considerably less inhibitory activity on insulin-induced oocyte maturation [2, 3]. These results suggested to us that oncogenic and activated wild-type ras-p21 proteins utilize overlapping but distinct signal transduction pathways [2, 3].

In support of this conclusion, we have found that oncogenic ras-p21 induces oocyte maturation using pathways that require a direct interaction with jun-N-terminal kinase (JNK) and its substrate, the transcriptional activation factor, jun [2, 3, 5, 6] and activation of the raf-MEK-MAPK pathway [4, 10, 11]. In contrast, insulin-activated wild-type ras-p21 appears not to depend completely on these two pathways. Thus, for example, we have identified glutathione-*S*-transferase, pi isozyme (GST-pi) as a selective inhibitor of JNK-induced phosphorylation of jun [12]. Co-injection of GST-pi with oncogenic ras-p21 into oocytes results in complete inhibition of oocyte maturation while this protein has no effect on insulin-induced maturation [13, 14]. In addition, the MAPK phosphatase, MKP-1T4, that de-activates this kinase, completely blocks oncogenic ras-p21-induced oocyte maturation but has considerably less effect on insulin-induced maturation [10].

Furthermore, we found that in oocytes induced to mature with oncogenic ras-p21, levels of phosphorylated JNK and MAPK rise strongly over the course of oocyte maturation [15]; these phosphorylations are blocked by both peptides. In contrast, phosphorylation of both kinases increases to much lower and constant levels, that do not correlate with extent of maturation, in oocytes that have been induced to mature with insulin [15].

In other studies, we identified possible sites at which each of the two ras peptides may act [2, 3]. We have found that PNC-2 blocks the oncogenic ras-p21–JNK interaction with a dose–response curve that coincides with that obtained for its inhibition of oocyte maturation [3]. This finding suggested that PNC-2 may block oncogenic ras selectively by inhibiting its interaction with JNK.

We have further found that PNC-7 blocks the interaction of oncogenic ras-p21 with raf [16]. Since both oncogenic and wild-type ras require raf activation, we surmised that each ras protein interacts with raf in differing ways resulting in differential activation of downstream kinases. Since both oncogenic ras-p21- and insulin-induced oocyte maturation are raf-dependent but only oncogenic ras-induced maturation requires raf activation of MEK and MAPK, we have been searching for possible alternate raf targets for the wild-type pathway.

We have recently found, using a complete *Xenopus* gene array, that insulin induces significantly higher levels of expression of two kinases in insulin-matured oocytes: lymphokine-activated killer T-cell-originated protein kinase (TOPK), a direct raf target [17], and dual-specificity-tyrosine-phosphorylation-regulated kinase-1A (DYRK1A), than in oncogenic ras-p21-matured oocytes [18]. Downregulation of these kinases with specific SiRNA's results in complete blockade of insulin-induced maturation but does not affect oncogenic ras-p21-induced maturation [19]. Thus, although raf activation is essential to signal transduction by

oncogenic and wild-type ras-p21, the pathways appear to branch downstream of raf [19].

All of these findings suggest that oncogenic and wild-type ras-p21, while sharing common targets, utilize different signal transduction pathways. An important implication of this conclusion is that, in ras-transformed cancer cells, oncogenic ras-p21 may be selectively blocked in such a way as to leave wild-type mitogenic signal transduction pathways intact.

The latter conclusion is supported by studies of the effects of these two peptides on a ras-transformed pancreatic cancer cell line, called TUC-3 [20]. These cells are derived from a rat pancreatic acinar cell line, called BMRPA1, which have been transfected with the ras-oncogene encoding Val 12–p21 protein. We have linked each of the two peptides to a penetratin sequence that allows them to cross the cell membrane and have incubated each with BMRPA1 and TUC-3 cells [20]. We found that both peptides induced complete reversion of TUC-3 cells to the untransformed phenotype within 2 weeks of incubation but had no effect on the viability or the growth of untransformed BMRPA1 cells [20]. These results suggest that both peptides block oncogenic ras-p21 selectively, and in cancer cells that possess intact wild-type ras or other regulated mitogenic pathways, these peptides allow for normal cell growth.

In this communication, we explore the effects of PNC-2 and PNC-7 on known ras-transformed human cancer cell lines to determine if they have similar effects as found for the TUC-3 cell line and to determine their potential efficacy against human cancers.

Materials and methods

Materials

Peptides

Four peptides, two ras-p21 and two control peptides, were synthesized by solid phase methods (Macromolecular Resources, Fort Collins, CO) [20]. Each peptide contained, on its carboxyl terminal end, the transmembrane-transporting penetratin peptide sequence, KKWKMRRNQFVK VQRG, called leader. All four peptides were shown to be >95% pure by mass spectroscopy. The sequences of these peptides are as follows:

1. ras-p21 96–110(PNC-2)-leader: YREQIKRVKDSDD VP-leader
2. ras-p21 35–47-leader: TIEDSYRKQVVID-leader
3. Control X13-leader (PNC-29) peptide: MPFSTGKRI MLGE-leader
4. Control CD45-leader peptide: NAVFRLLHEHKG KKA-leader

The X13 sequence in control sequence 3 is from cytochrome P450 [20]. In addition, for the LDH assays for tumor cell necrosis (see below), we employed another control peptide, PNC-28, known to induce tumor cell necrosis [21], whose sequence is ETFSDLWKLL-leader.

Cell lines

HT1080 (human fibrosarcoma) and MIAPaCa-2 (human pancreatic cancer) cell lines were obtained from the American tissue type and cell collection (ATCC) (Manassas, VA); U-251 (human astrocytoma) cells were the kind gift of Dr. D. Weinstin (GliaMed, NY) [11].

Methods

Cell cultures. MIAPaCa-2

Cells were grown in Dulbecco's modified Eagle's medium (GIBCO) supplemented with heat inactivated 10% calf or fetal calf serum, 20 mM glutamine, penicillin (100 units/ml), streptomycin (100/ug/ml) and plated on a six-well plate with density no more then 20,000 cells/well in 3 ml culture medium. *HT1080* cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 units/ml of penicillin and 100 ug/ml streptomycin and plated on six-well plates with a density of no more then 6,000 cells/well in 3 ml culture medium. BMRPA1 rat pancreatic acinar cells were grown as described previously [20].

Incubation of peptides with cells

All cells (approximately 2×10^4) were incubated in their respective media (see above) for 24 h after which the media were replaced with the same media containing one of the peptides at a concentration of 100 μg/ml. Over the subsequent time course of each experiment, the medium was then changed every 48 h and always contained peptide at the same concentration. Cells were observed daily for 1–3 weeks for changes in morphology and growth characteristics. After selected time periods, cells were stained with trypan blue or assayed using the MTT assay as described previously [11, 19] to assess cell counts. These experiments were performed at least three times for each cell line.

Growth of cells in soft agar [20]

To assess whether cells that appeared by morphology to have reverted to the untransformed phenotype after treatment

with a peptide, we assessed whether the cells would grow on soft agar. In these experiments, performed three times, the cells were isolated, replated, allowed to grow to confluence in 10% FCS-DMEM and then trypsinized. At this time, 1×10^4 cells were then mixed with 0.37% bactagar (Difco Laboratories, USA) and then added to culture plates containing solidified 0.6% bactagar medium. After the agar solidified, the plates were incubated at 37°C for 2 weeks after which they were subjected to cell counts using the MTT assay. Colony counts were obtained directly from observing the agar.

Caspase and LDH assays

To detect if peptide-induced cell death occurred by apoptosis or necrosis, we performed the Clontech (Palo Alto, CA) caspase (CPP32) activity assay for elevated caspase as described previously [21]. In addition, to detect if significant cell necrosis occurred, we used the CytoTox96 assay (Promega, Madison, WI) for LDH released into the cell culture medium as also described previously [21]. As a positive control for LDH release, the CytoTox96 assay was performed on the media of MIA-PaCa-2 cells that were incubated with 100 ug/ml PNC-28 peptide for 24 h [21].

Immunoblots for total and phosphorylated JNK

Blots for total and phosphorylated JNK were performed as described previously [11, 15] for the following cells: HT1080 prior to incubation and after 2 weeks incubation with PNC-2-leader and PNC-7-leader; and MIA-PaCa-2 cells prior to incubation and after 2 weeks incubation with these two peptides. Approximately, 2×10^6 cells were twice washed with cold PBS and lysed by adding the lysis buffer [15]. Samples of lysates containing constant amounts of protein (either 50 or 75 μg) were subjected to SDS PAGE on a 12% resolving gel and the proteins then electrophoretically transferred onto nitrocellulose membranes overnight at 4°C; the membranes were then blocked with non-fat dry milk in Tris-buffered saline with 1% Tween-20 (TBS-T) (pH 7.6) and were then incubated with the appropriate anti-kinase antibodies as follows: (1) anti-JNK polyclonal antibody (Sigma, St Louis, MO) which recognizes both JNK-1 and JNK-2, diluted 1:1,000; (2) anti-phospho JNK (JNK-P) phosphorylated at positions Thr 183 and Tyr 185 (Promega, Madison, WI), diluted 1:800. All incubations were performed for 12 h at 4°C, after which the membranes were washed three times with TBS-T and incubated with anti-rabbit secondary antibody (Amersham, Piscataway, NJ) at 1:4,000 dilution. Detection was accomplished using the ECL chemiluminescence detection kit (Amersham) [11, 15].

Results

Effects of PNC-2 and PNC-7 on ras-transformed HT-1080 fibrosarcoma cells

HT-1080 is a ras-transformed fibrosarcoma cell line. Figure 1 shows that, when we incubated these cells with either PNC-2- or PNC-7-leader peptides, after a small increase in tumor cell number up to 48 h, there was a dramatic decrease in cell count after 1 week. This count stabilized below the initial level and remained stable thereafter for a three-week observation period. As also shown in Fig. 1, control peptides such as X13-leader (PNC-29) and CD-45-leader, had no effect on tumor cell growth which was the same as for untreated cells (Fig. 1). In addition, as we reported previously [20], incubation of BMRPA1 cells with PNC-2-leader, PNC-7-leader or PNC-29 control peptide had no effect either on cell growth or viability (see ref. [20]). These results suggest that inhibition of tumor cell growth by both PNC-2- and PNC-7-leader peptides is peptide-specific and that the growth-inhibitory effects of these peptides is specific for cancer cells.

Since the cell count stabilized for HT-1080 cells that were treated with PNC-2 or PNC-7 (Fig. 1), we explored whether the cells may have reverted to the untransformed phenotype as we found for TUC-3 cells treated with each of these peptides [20]. We therefore plated these treated cells in soft agar. As a control, we likewise plated control peptide-treated cells in soft agar. Figure 2 shows that the control peptide-treated cells form multiple colonies while the cells treated with either of our ras peptides do not form any colonies. This result suggests that both peptides induce loss of a critical characteristic of transformed cells. Coupled with our finding that both peptides block the growth of these cells, we conclude that our peptides induce phenotypic reversion of this cell line to non-transformed cells.

Effect of ras peptides on MIA-PaCa-2 pancreatic cancer cells

Since we found that both of our ras peptides induce complete phenotypic reversion of the TUC-3 rat pancreatic cancer cell line [20], and since oncogenic ras is known to be a major causative factor in over 90% of human pancreatic cancers [2], we extended our study to a ras-transformed human pancreatic cancer cell line, MIA-PaCa-2. As shown in Fig. 3, both peptides, but not the negative control peptide, induce total inhibition of tumor cell growth. When we examined the cells after 2 weeks of treatment with either peptide, we found that no viable cells remained, i.e., there was total cell death (not shown). In order to explore the general cause of tumor cell death, we assayed the cells for caspase, a marker for apoptosis [21, 22]. In addition, we

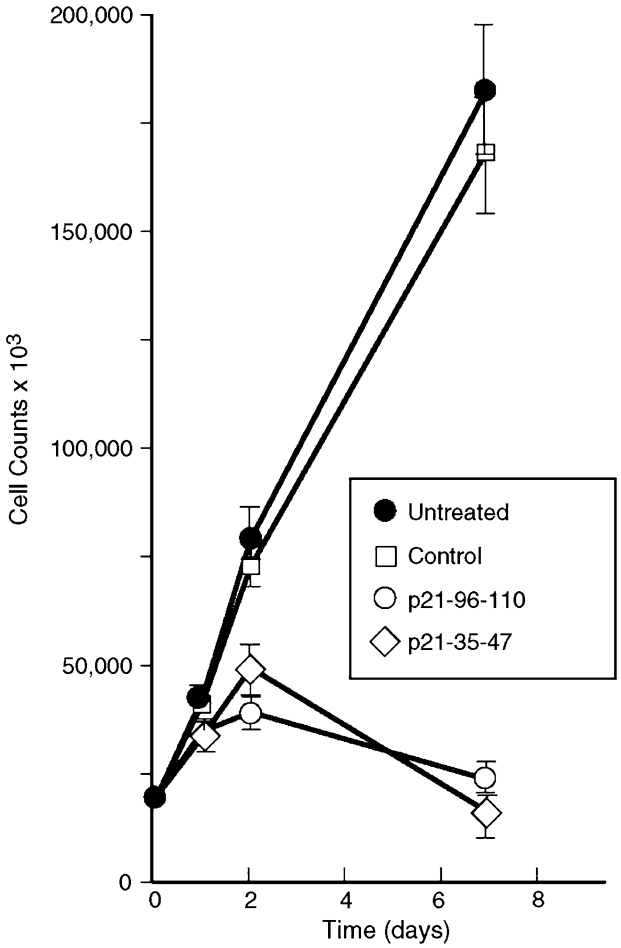

Fig. 1 Effects of PNC-2-leader (labeled p21 96–110) and PNC-7-leader (labeled p21 35–47) on the growth of HT-1080 cells. Control was CD-45-leader peptide. All *symbols* are explained in the *inset*

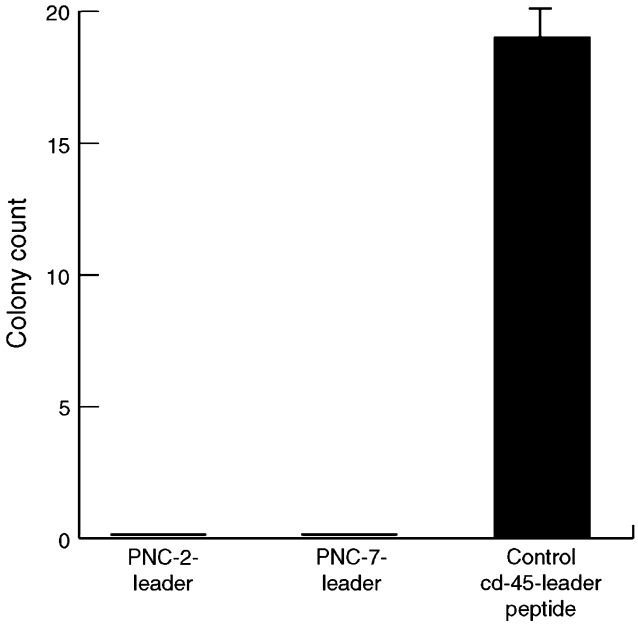

Fig. 2 Colony counts for HT-1080 cells treated with PNC-2-leader and PNC-7-leader peptides grown in soft agar. The positive control was HT-1080 cells treated with CD-45-leader peptide

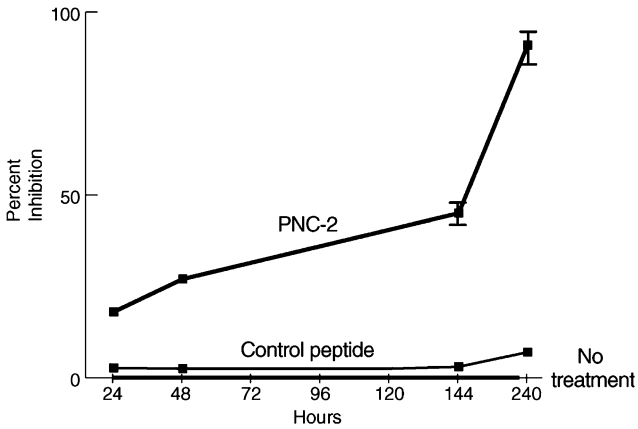

Fig. 3 Effects of PNC-2 on cell growth of MIA-PaCa-2 cells. After 10 days, the cell count dropped to 0. Control was the CD-45-leader peptide

assayed the culture medium for lactate dehydrogenase (LDH) as a marker for tumor cell necrosis [21, 23]. As shown in Fig. 4a, caspase expression, while markedly elevated in cells treated with TNF-alpha that is known to induce apoptosis [24], is not elevated above control background levels in cells treated with either ras-derived peptide. On the other hand, as shown in Fig. 4b, LDH is released from cells treated with either ras peptide but is two-fold lower in the media of untreated cells or cells treated with PNC-29 control peptide (not shown). These results suggest that both PNC-2- and PNC-7-leader peptides induce tumor cell necrosis of ras-transformed MIA-PaCa-2 cells.

Effects of peptide treatment on JNK expression and phosphorylation

In prior studies on oocytes, we found that, in oocytes that were induced to mature by microinjection of oncogenic ras-p21, there was an early, strong induction of JNK and MAPK phosphorylation that increased as maturation progressed [15]. In contrast, in oocytes induced to mature with insulin, that activates wild-type ras-p21 [9], phosphorylation of JNK became observable at a later time and remained at a much lower level than that observed in oncogenic ras-p21-matured oocytes [15]. When we co-injected oncogenic ras-p21 together with either of the two ras peptides, maturation was blocked and phosphorylation of both JNK and MAPK was strongly diminished [15].

Since both peptides induce tumor cell necrosis of ras-transformed MIA-PaCa-2 cells, we sought to determine if they cause diminished phosphorylation of JNK as we observed in the oocyte system. As shown in Fig. 5, expression

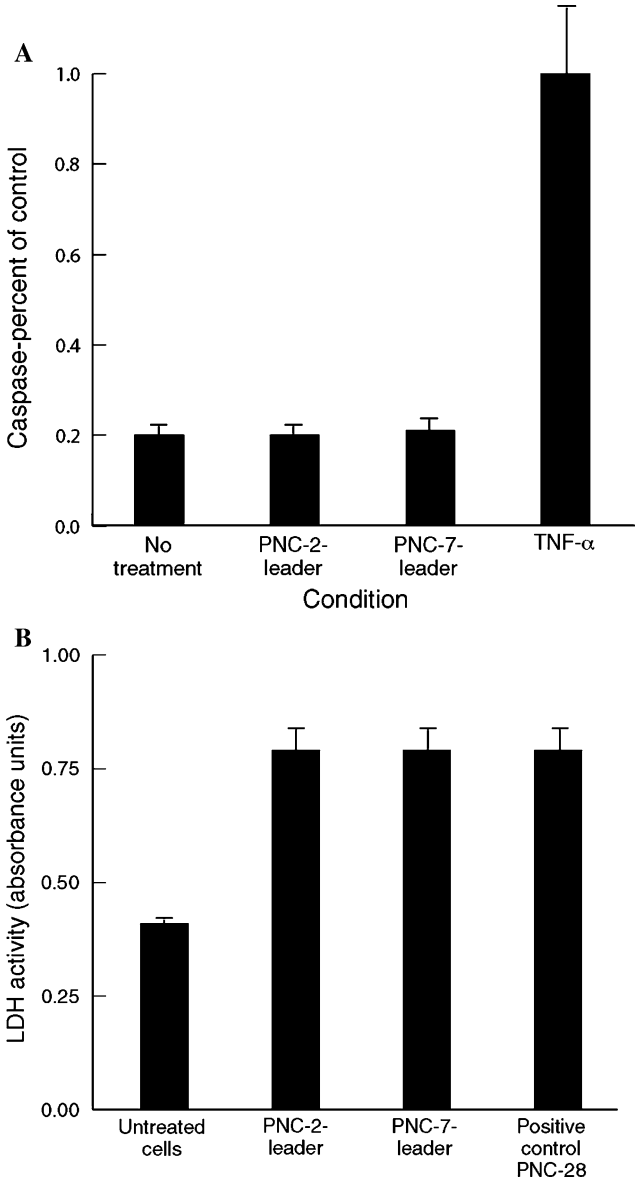

Fig. 4 **a** Caspase expression as fraction of positive control (TNF-alpha-treated cells) in MIA-PaCa-2 cells treated with PNC-2-leader and PNC-7-leader peptides. Controls were untreated MIA-PaCa cells (negative control) and MIA-PaCa-2 cells incubated for 45 min with tumor necrosis factor (TNF)-alpha (Sigma) (20 ng/ml), known to induce apoptosis. **b** LDH activity in medium for MIA-PaCa-2 cells: untreated (condition 1); treated with PNC-2-leader (condition 2); treated with PNC-7-leader (condition 3); treated with positive control PNC-28 peptide (21) (condition 4)

of phosphorylated JNK is high in the untreated cells. In cells treated with either PNC-2 or PNC-7, while the expression of total JNK remains the same as in untreated cells, the level of phosphorylated JNK is markedly diminished. These results suggest that both peptides block activation of JNK by oncogenic ras-p21 that may be a causative factor in tumor cell death.

Discussion

The two ras peptides are selective for blocking oncogenic ras-pathways

Our results with PNC-2 and PNC-7 in *Xenopus* oocytes suggested that these peptides block oncogenic ras-p21 but leave intracellular functioning of wild-type ras-p21 intact. Based on these results, we surmised that in ras-transformed cancer cells, that were heterozygous for oncogenic ras-p21 expression, these peptides might block oncogenic ras-p21 selectively and leave the wild-type signal transduction pathways intact. If cancer cells were homozygous for oncogenic ras-p21, then blockade of this protein might block cell growth completely, leading to cell death.

When we tested each peptide, linked to a membrane-translocating penetratin sequence, against TUC-3 cells that are stably ras-transformed BMRPA1 pancreatic cells that therefore contain both wild-type and oncogenic forms of ras-p21, we found that the cells reverted to the untransformed phenotype supporting our hypothesis [20]. Since this result was reproduced in the same cells transfected with plasmids encoding either peptide, we concluded that the ras-p21 sequences themselves induced reversion [20].

Because these were non-human cells that were "models" in the sense that they were produced by stably transfecting with oncogenic k-*ras* the untransformed pancreatic acinar BMRPA1 cell line, we next sought to determine the effects of these peptides on known ras-transformed human cancer cell lines. In this study, therefore, we tested each peptide against three ras-transformed human cancer cell lines and found that each ras peptide, but not either control peptide, induces arrest of cancer cell growth.

The two ras peptides also block human ras-transformed cancer cell growth

We now find that these peptides block growth and induce phenotypic reversion of HT1080 fibrosarcoma and induce cancer cell necrosis of MIA-PaCa-2 pancreatic cancer cells. These results suggest that both PNC-2-leader and PNC-7-leader peptides block growth of ras-transformed human cancer cells. The abilities of these peptides to block human cancer cell growth are specific since control peptides, that contain the same penetratin leader sequence on their respective carboxyl termini, have no effect on the growth of these cancer cell lines. The results with the control peptides further suggest that the leader sequence itself is not responsible for the observed growth-inhibitory effects of PNC-2-leader and PNC-7-leader. In addition, these peptides exert their growth-inhibitory effects only on cancer cells since neither peptide is cytotoxic to untransformed cells in culture including the BMRPA1 epithelial cell line.

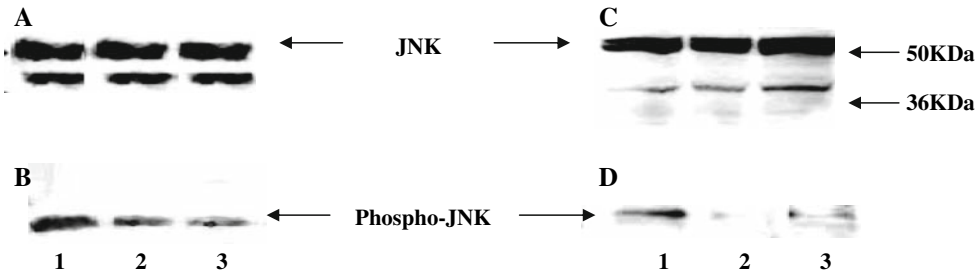

Fig. 5 Blots for the expression of JNK and phospho-JNK in both MIA-PaCa-2 (**a**, **b**) and HT-1080 cells (**c**, **d**) treated with PNC-2-leader and PNC-7-leader peptides. **a** All lanes blotted for total JNK: *lane 1* untreated MIA-PaCa-2 cells. *Lane 2* MIA-PaCa-2 cells treated for 48 h with PNC-2-leader. *Lane 3* MIA-PaCa-2 cells treated for 48 h with PNC-7-leader. **b** All lanes blotted for phospho-JNK as described in the "Methods". *Lane 1* untreated MIA-PaCa-2 cells. *Lane 2* MIA-PaCa-2 cells treated for 48 h with PNC-2-leader. *Lane 3* MIA-PaCa-2 cells treated for 48 h with PNC-7-leader. **c** All lanes blotted for total JNK: *lane 1* untreated HT-1080 cells. *Lane 2* HT-1080 cells treated for 48 h with PNC-2-leader. *Lane 3* HT-1080 cells treated for 48 h with PNC-7-leader. **d** All lanes blotted for phospho-JNK as described in the "Methods". *Lane 1* untreated HT-1080 cells. *Lane 2* HT-1080 cells treated for 48 h with PNC-2-leader. *Lane 3* HT-1080 cells treated for 48 h with PNC-7-leader

Mechanisms for peptide induction of cancer cell growth arrest

As shown in Fig. 2, both PNC-2-leader and PNC-7-leader peptides induce phenotypic reversion of ras-transformed HT-1080 human fibrosarcoma cells as revealed by the inability of treated cells to grow on soft agar. This result correlates well with our previous results on k-*ras*-transformed TUC-3 rat pancreatic cancer cells. After 2 weeks of treatment of these cells with either peptide, the cells were found to adopt the wild-type phenotype; unlike untreated TUC-3 cells, these treated cells failed to grow in nude mice [20].

On the other hand, surprisingly, both peptides induced cell death, and not the phenotypic reversion, of MIA-PaCa-2 human pancreatic cancer cells. This result suggests either that there may be a change in the mechanism of inhibition, by these peptides, of cancer cell growth in this cell line or that this cell line may lack alternate "rescue" pathways allowing for normal cell growth.

To understand further the mechanism of peptide-induced MIA-PaCa-2 cell death we induced by these peptides, we explored whether these peptides may induce apoptosis of these cells. One of the main targets of activation cascades in apoptosis are the caspases [22]. Thus, we inquired whether these peptides induced high caspase expression in this cell line. As shown in Fig. 4a neither peptide induces increased caspase expression in this cell line, eliminating this mechanism for induction of cell death. In other studies on peptides derived from p53 [21], we have found that these peptides induce tumor cell necrosis evidenced by release of the cytosolic enzyme, LDH, into the medium due presumably to cell membrane breakdown. As shown in Fig. 4b, both peptides induce the release of high levels of LDH into the medium suggesting that both peptides induce tumor cell necrosis.

Effects of the two ras peptides on JNK activation

In our prior studies in oocytes on oncogenic and insulin-activated wild-type ras-induced oocyte maturation, we found that oncogenic ras-p21 induces maturation of oocytes that have high levels of expression of phosphorylated JNK and MAPK (ERK) that are not observed in insulin-matured oocytes [15]. When we co-injected Val 12–ras-p21 together with either PNC-2 or PNC-7, we observed that phosphorylation of these kinases was strongly downregulated in the oocytes [15]. These results were consistent with our prior findings that oncogenic ras-p21 can directly activate JNK and that PNC-2 blocks this interaction [6, 7, 13, 14].

As shown in Fig. 5, HT1080 and MIA-PaCa cells have high levels of phosphorylated JNK as we originally found in oocytes that were microinjected with oncogenic ras-p21 [15]. As we also found in oocytes, both peptides cause large decreases in phosphorylated JNK in both HT1080 and MIA-PaCa-2 ras-transformed human cancer cell lines. Since oncogenic ras appears to require JNK as a critical downstream target and since both PNC-2 and PNC-7 block its activation, we surmise that this blockade is critical to the inhibitory effects of both peptides.

If blockade of this pathway lies in common to the effects of these peptides in both HT1080 and MIA-PaCa-2 cells, the question arises as to why these two cell lines respond differently to these peptides, i.e, phenotypic reversion or necrosis, respectively. One possible difference may lie in the presence of alternate or "rescue" pathways. If both peptides block signal transduction by oncogenic ras, continued cell growth would then depend on the availability of alternate pathways such as the TOPK/DYRK1A pathway in oocytes [19]. HT1080 cells are known to be heterozygous for oncogenic ras. If wild-type ras in these cells can function via alternate pathways such as may be available in the oocyte system or ones similar to them, normal cell growth

may result. On the other hand, if such pathways are lacking, such as may exist in homozygously ras-transformed cells, all cell growth may cease, leading to cell death. Since MIA-PaCa-2 cells are homozygous for oncogenic ras-p21, this latter phenomenon may take place as a result of peptide treatment.

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