IL24 and its Receptors Regulate Growth and Migration of Pancreatic Cancer Cells and Are Potential Biomarkers for IL24 Molecular Therapy

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Abstract. *Background: Pancreatic cancer is hard to diagnose and treat due to its asymptomatic development and early metastasis. Supplementary therapy including molecular targeted therapy is needed to improve the outcome of pancreatic cancer. The significance of interleukin 24 (IL24) and its receptors in pancreatic cancer were investigated in this study. Materials and Methods: Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was carried out in 200 patient samples of pancreatic cancer. Transcript and protein expression were investigated in pancreatic cancer cells. Impact of IL24 recombinant protein on cell functions was examined. Results: High IL20R1 transcript expression was related to early T stage, and advanced N, and M stage. They collectively correlated with the survival of the patients. Treatment with IL24 inhibited cell growth, but its impact on migration varied depending on protein concentration. Conclusion: IL20R1 correlated with prognosis of patients with pancreatic cancer, and mediates pancreatic cancer cell growth and migration. It may be a potential biomarker for IL24 molecular-targeted therapy.*

Pancreatic cancer is the seventh most frequent tumour in Europe, but it ranks as the fourth and fifth leading cause of cancer death in America and Europe, respectively. (1). Surgery, chemotherapy and radiotherapy are the three main types of treatment for pancreatic cancer; currently only radical surgery may provide curative treatment. However, the frequency of diagnosis at a late stage leads to only a small percentage of patients with early-stage disease, and radical surgery is restricted to 10-20% of patients diagnosed with stage I or II pancreatic cancer; even worse, the overall 5-year survival rate of early-stage patients is only 10% (2, 3). Combined therapy is essential in the treatment of pancreatic cancer, for early metastasis during carcinogenesis, neoadjuvant chemotherapy and adjuvant chemoradiotherapy were indispensable when curative surgery was adopted (4-7).

Molecular targeted therapy has also been adopted in the management of pancreatic cancer, and better effect was gained through the combination of the small-molecular tyrosine kinase inhibitor erlotinib with gemicitabine-based chemotherapy (8-11).

In this study, we focused on another promising candidate for molecular targeted therapy. Interleukin 24 (IL24) belongs to the IL10 subfamily of cytokines, and was found to induce apoptosis selectively in various cancer cell lines and xenograft mouse models (12-16). A Phase I clinical study using non-replicating adenoviral vector expressing IL24 have previously been carried out with promising results (17). Both IL20R1 and IL22R1 are receptor subunits of the IL10 family, and join together with IL20R2 to construct a heterodimeric receptor complex. The two heterodimeric receptors IL20R1/IL20R2 and IL22R1/IL20R2 are receptors shared by both IL24 and IL20 (18, 19). A study of transgenic mice overexpressing IL20 indicated that IL20 is involved in regulation of skin abnormalities (20). Hence given the potential for IL24 to induce apoptosis in a variety of cancer cells and its early success in phase I trials our current study

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Table I. *Primers sequences used in the current study.*

Primer	Forward	Reverse
IL-20R1	TACAATGGACTCCACCAGAG	ACTGAACCTGACCGTACATATTCAGCCATTTCTTTTGC
IL-20R2	GCCTGGAGAAACAGTGTACT	ACTGAACCTGACCGTACACAGGACCTTCAGTGAGTGAG
IL-22R1	AGATGACTGACAGGTTCAGC	ACTGAACCTGACCGTACAGAATCGATCTCACTTTGGAG
IL-24	GATGTTTTCCATCAGAGACAG	ACTGAACCTGACCGTACACATCCAGGTCAGAAGAATGT

investigated the significance and potential of this cytokine and it's receptors in pancreatic cancer. In the current study the expression of IL24, IL20R1, IL20R2 and IL22R1 were investigated in a clinical cohort of patients with pancreatic cancer and pancreatic cancer cell lines, and their correlation and potential role in pancreatic cancer was explored.

Materials and Methods

Materials. PANC 1, MIA PACA2 and ASPC cell lines were obtained from the European Collection of Cell Cultures (Salisbury, Wiltshire, UK). Secondary antibodies and monoclonal/polyclonal antibodies raised against a peptide mapping at the N-terminus of human IL24, IL20R1, IL20R2, IL22R1, IL10R1 and IL10R2 were purchased from Santa Cruz Biotechnologies Inc. (Santa Cruz, CA, USA).

Fresh-frozen pancreatic adenocarcinoma tissues (n=202), along with matched normal tissue from the same patients, were collected immediately after surgical resection at the Beijing Cancer Hospital and were stored at the Tissue Bank of Peking University Oncology School. Clinicopathological factors, including age, sex, histological type, TNM stage, and lymph node metastasis, were recorded and stored in the patient database. All protocols were reviewed and approved by the local Ethics Committee (MTA01062008) and consent was obtained from the patients. Patients were recruited between January 2002 and December 2009 and were routinely followed up.

Tissue processing, RNA extraction, cDNA synthesis and reverse transcription-polymerase chain reaction (RT-PCR). Frozen sections of tissue were cut to thicknesses of 5-10 μm and kept for immunohistochemistry and routine histology. RNA was isolated using Total RNA Reagent (Promega Corporation, Madison, WI, USA) (21). cDNA synthesis and RT-PCR were performed using standard methods.

Quantitative analysis of gene transcripts. The mRNA expression of *IL24, IL20R1, IL20R2,* and *IL22R1* in pancreatic cancer were determined by real-time quantitative (q) RT-PCR, based on Amplifluor™ technology as previously described (22, 23). qRT-PCR primers were designed using Beacon Design software (PREMIER Biosoft, Palo Alto, CA, USA). The underlined sequence of the primers used in this study shown in Table I is complementary to the universal Z probe (TCS Biologicals Ltd., Oxford, UK).

Western blot analysis. The protein concentration in cell lysates was determined using the DC Protein Assay kit (Bio-Rad Lifesciences Company, Hercules, CA, USA) and an ELx800 spectrophotometer (Bio-Tek, Swindon, UK). Proteins were probed with antibodies to IL24, IL20R1, IL20R2, IL22R1, IL10R1, IL10R2 (all 1:200), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:500) (Santa Cruz Biotechnologies Inc.) as internal control, followed by a peroxidase-conjugated secondary antibody (1:1,000). Protein bands were visualized and photographed using an UVITech imager (UVITech, Ltd., Cambridge, UK).

In vitro cell-growth assay. Cells were plated into 96-well plates at 2,500 cells/well, with a concentration gradient of recombinant IL24 protein at 1:2 dilution in medium, the dilution starting from 125 ng/ml. Cell growth was assessed after 1, 3 and 5 days. Crystal violet was used to stain cells and absorbance was determined at a wavelength of 540 nm using a spectrophotometer (ELx800; Bio-Tek).

Cell-matrix adhesion assay. The cell-matrix adhesion assay was carried out as previously described (24). Cells were added to a 96-well plate precoated with Matrigel (BD Biosciences, Oxford, UK) (5 μg/well), with a concentration gradient of recombinant IL24 protein at 1:2 dilution in medium, the dilution starting from 125 ng/ml. After 40 min of incubation, non-adherent cells were washed off using phosphate buffered saline. The remaining cells were fixed, stained and counted.

Wounding/migration assay. The wounding assay was performed as previously described (25). A monolayer of cells was scraped with a blunted fine-gauge needle. The movement of pancreatic cancer cells to close the wound under treatment of 31 ng/ml recombinant IL24 protein was recorded on a time-lapse video recorder and analyzed using Optimas 6.0 motion analysis software (Meyer Instruments, Houston, TX, USA).

In vitro invasion assay. The *in vitro* invasion assay was carried out as previously described (25). Transwell inserts with 8 μm pore size were coated with 50 μg of Matrigel (Collaborative Research Products, Bedford, MA, USA) and air-dried. Following rehydration, 40,000 cells were added to each well, recombinant IL24 protein was added into the lower medium at 31 ng/ml. After 3 days' incubation, cells that had migrated through the matrix to the other side of the insert were fixed, stained and counted.

Electric cell-substrate impedance sensing (ECIS)-based cell adhesion assay. The ECIS Z-Theta instrument and 96W1E arrays (Applied Biophysics, Inc., Troy, NY, USA) were used herein, as recently reported (26). Briefly, the same number of cells (60,000) was seeded to each well of the ECIS arrays. Recombinant IL24 protein was added at 62, 31 and 16 ng/ml. Impedance and resistance of the cell layer was immediately recorded for a period of up to 20 hours. When the cells had reached confluence, the monolayer in each well was electrically wounded using the manufacturer's wounding function (6V, 3,000 μA, and 6,000 Hz for 30 sec) to

Table II. *Correlation of IL20R1 transcript expression and clinicol-pathological parameters*.

	n	Median	*p*-Value	Q1	Q3
Tissue					
Tumour	184	664.2	<0.001	212	1458
Normal	145	140.1		42	480
Gender					
Male	116	671	0.4168	228	1482
Female	68	663.2		154	1424
Differentiation					
Highly differentiation	11	750.1	0.333	94	2997
High and moderate differentiation	13	1317.3		291	2536
Moderate differentiation	65	630.3		145	1327
Moderate and poor differentiation	72	792.6		256	1673
Poor differentiation	11	520.3		89	1165
Vascular invasion					
Yes	50	693.7	0.7749	234	1286
No	108	886.9		217	1637
T stage					
1	4	46.45	0.002	0	1275
2	25	1726.08		550	2402
3	101	591.12		145	1162
4	22	767.82		282	1629
Tumour invasion					
1~2	29	1507.7	0.0153	197	2306
3~4	123	617.8		196	1239
N Stage					
Negative	72	340.5	0.3	41	1375
Positive	4	465		0	1275
Distant metastasis					
Yes	15	737.3	0.7157	161	1944
No	169	659.4		213	1429
TNM stage					
1~2	133	682.6	0.7798	210	1453
3~4	29	634.3		262	1722
Survival					
Alive	42	738.6	0.7057	188	1691
Death	128	705.4		230	1438

Table III. *Correlation of IL20R2 transcript expression and clinicol-pathological parameters*.

	n	Median	*p*-Value	Q1	Q3
Tissue					
T	199	58.79	<0.001	12	266
N	146	3.54		0.92	13.55
Gender					
Male	120	49.7	0.568	11	249
Female	79	85.4		13.8	267.5
Differentiation					
Highly differentiation	12	38.25	0.99	23	262
High and moderate differentiation	16	124.1		35.8	179.3
Moderate differentiation	68	62.93		9.8	267.1
Moderate and poor differentiation	76	60.68		11	3423
Poor differentiation	12	52.09		24.7	279
Vascular invasion					
Yes	50	693.7	0.7749	234	1286
No	108	886.9		217	1637
T stage					
1	5	105.75	0.224	39.6	133.1
2	27	65.37		16	351
3	111	45.46		9.1	176
4	22	213.5		13	506
Tumour invasion					
1~2	32	78.3	0.3789	17	260
3~4	133	49.6		10.6	266.8
N Stage					
Negative	80	55.2	0.8739	12	183.4
Positive	5	105.7		39.6	133.1
Distant metastasis					
Yes	15	147	0.1291	15	675
No	184	55.2		11.5	234.01
TNM stage					
1~2	146	49.7	0.0174	11.1	177.2
3~4	29	266.2		18	624
Survival					
Alive	44	41.1	0.4939	18	185
Death	139	85.4		12.1	350.9

create a 250-μm wound per well. Subsequent changes in resistance, noted as the wounded cells migrated in to close this wounded area, were recorded for a period of up to 20 h. Data were analysed using the ECIS software, supplied by the manufacturer.

Statistical analysis. Statistical analysis was performed using SPSS software (SPSS Standard version 13.0; SPSS Inc., Chicago, IL, USA). The relationship between IL24 receptor subunit expression and tumour grade, TNM staging and nodal status was assessed using Mann–Whitney *U*-test and Kruskal–Wallis test. Survival over the follow up period was analysed using Kaplan–Meier survival analysis. Differences were considered statistically significant at $p<0.05$.

Results

Transcript expression of IL24 and its receptors in pancreatic adenocarcinoma tissues. Transcript expression of IL24 and its receptors were examined in specimen of 200 patients with pancreatic adenocarcinoma using real-time RT-PCR (expressed as mean IL24/IL24 receptors transcript copies/μl of RNA from 50 ng total RNA and standardized with *GAPDH*). The cohort comprised of 121 men (60.5%) and 79 women (39.5%). The average age of patients was 60.69 years. The median follow-up time was 11.98 (range=1-112) months, 153 patients died of pancreatic cancer, patients who died of other causes were excluded, and 46 remained alive and disease-free by the end of the study.

No detectable amounts of *IL24* transcript were observed in most pancreatic cancer tissues and qRT-PCR analysis, for these samples did not cross the threshold. The *IL20R1* mRNA level was the highest among IL24 receptor subunits. *IL22R1* transcript expression was the lowest; expression of *IL20R2* transcript was between that of the IL24 receptor subunits (Tables II-V).

Table IV. *Correlation of IL22R1 transcript expression and clinicol-pathological parameters.*

	n	Median	*p*-Value	Q1	Q3
Tissue					
T	109	1.86		0.36	7.76
N	146	4.45		0.71	11.61
Gender					
Male	68	2.84		0.57	9.06
Female	41	0.55		0.2	4.9
Differentiation					
Highly differentiation	2	6.65		*	*
High and moderate differentiation	9	1.39		0.25	16.15
Moderate differentiation	43	1.78		0.46	6.94
Moderate and poor differentiation	43	2.13		0.41	8.593
Poor differentiation	8	0.5		0.2	59.8
Location					
1	25	2.1		0.57	7.747
2	2	0.181		*	*
3	15	1.39		0.33	6.947
4	0	*		*	*
5	1	0.005		*	*
Vascular invasion					
Yes	59	2.1		0.33	10.04
No	31	1.32		0.46	6.63
T stage					
1	2	3.47		*	*
2	10	1.164		0.116	2.531
3	67	1.86		0.39	9.16
4	13	2.73		0.44	5.79
Tumour invasion					
1~2	12	1.164		0.31	7.11
3~4	80	2.15		0.43	9.78
N Stage					
Negative	43	1.06		0.27	5.5
Positive	2	3.47		*	*
Distant metastasis					
Yes	9	28.77		0.91	47.77
No	100	1.72		0.34	6.61
TNM stage					
1~2	80	1.82		0.31	7.11
3~4	18	2.42		0.43	12.98
Survival					
Alive	28	1.45		0.43	9.78
Death	75	2.1		0.27	8.34

Table V. *Correlation of IL24 transcript expression and clinicol-pathological parameters.*

	n	Median	*p*-Value	Q1	Q3
Tissue					
T	173	0		0	24
N	169	0		0	0
Gender					
Male	107	0	0.8637	0	19
Female	66	0		0	56
Differentiation					
Highly differentiation	11	0.1	0.569	0	1.6
High and moderate differentiation	16	0		0	83
Moderate differentiation	58	0		0	2
Moderate and poor differentiation	66	0		0	61
Poor differentiation	9	3		0	207
Vascular invasion					
Yes	48	0	0.3132	0	21
No	100	0		0	57
T stage					
1	4	0.58	0.762	0	12.25
2	26	0		0	62.8
3	93	0		0	35
4	21	0		0	4
Tumour invasion					
1~2	30	0	0.2657	0	23.6
3~4	114	0		0	19
N Stage					
Negative	74	0	0.7392	0	23
Positive	4	0.58		0	12.25
Distant metastasis					
Yes	12	0	0.9805	0	21.4
No	161	0		0	2.4
TNM stage					
1~2	125	0	0.5588	0	37
3~4	27	0		0	12
Survival					
Alive	37	0	0.8599	0	24
Death	120	0		0	50

Correlation of IL24 receptor expression with tumour invasiveness and clinical staging. The relation of IL24 receptor expression and clinicopathological parameters was also assessed in our study through quantitative analysis of IL24 receptor transcripts.

All the subunits were significantly differently expressed between tumour and normal tissues. Significantly higher expression of IL20R1 was observed in T1-2 stage tumours (p=0.015). IL20R2 also presented higher transcript expression in T1-2 stage, while IL22R1 transcript expression was lower in T1-2 stage compared to T3-4 stage. The transcript expression of these subunits were consistent in relation to lymph node metastasis, with metastasis positivity of lymph nodes corresponding to higher transcript expression. Higher expression of these subunits also corresponded to the presence of distant metastases, but the difference was not significant. The expression was consistent in relation to both TNM stage and survival: higher expression of IL20 was correlated with lower TNM stage and "alive" survival status (Figure 1). On the contrary, high expression of IL20R2, and IL 22R1 were related to advanced TNM stage and death, IL20R2 expression was significantly lower in TNM1-2 stage patients compared with TNM3-4 stage patients (p=0.0174) (Tables II-V).

The levels of expression of IL24 and the receptors were analyzed according to survival of the patients. Individually,

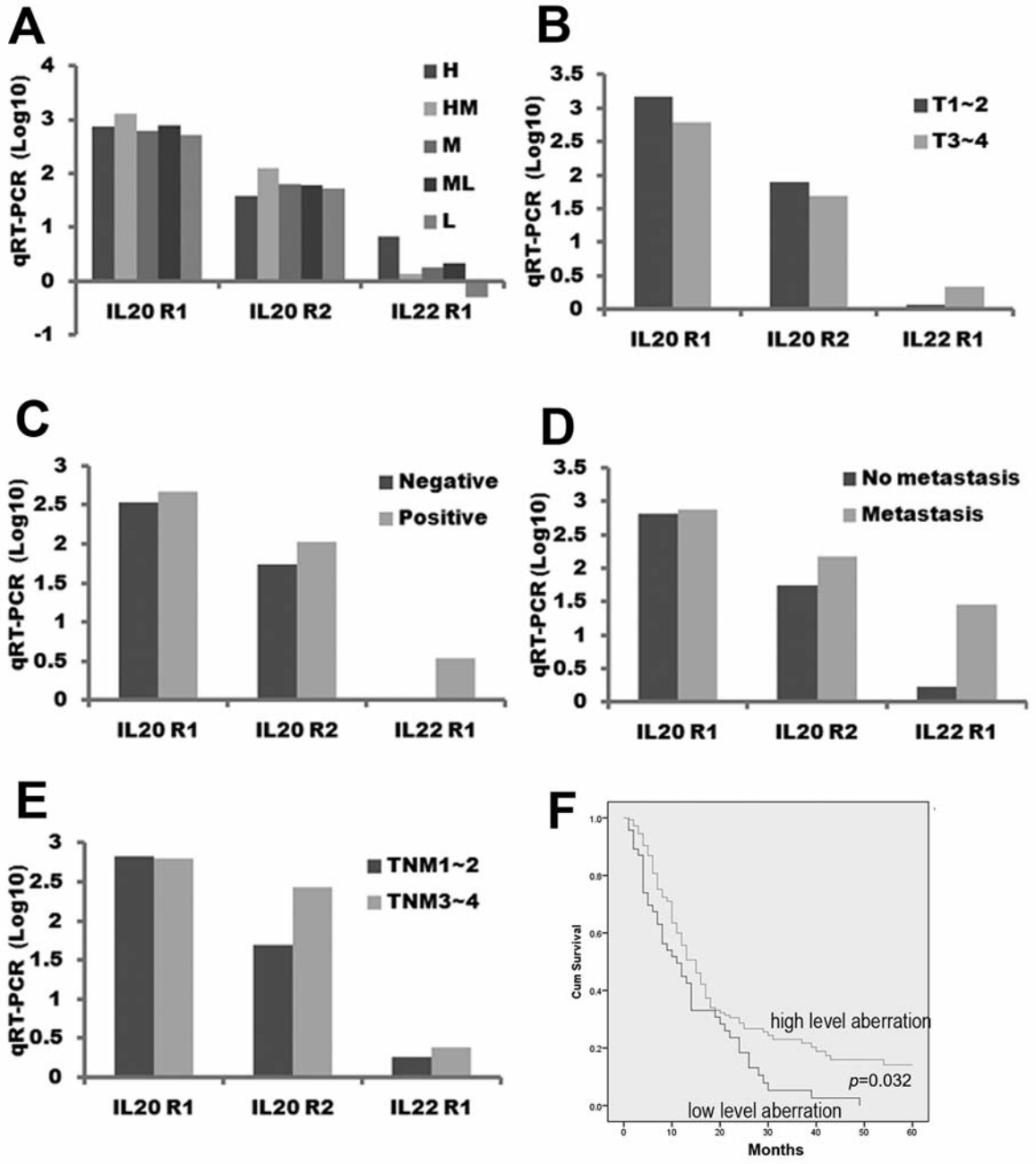

Figure 1. *Correlation of interleukin 24 (IL24) receptor subunits in the pancreatic cancer cohort according to clinicopathological parameters: A: differentiation grade; B: T stage; C: lymph node metastasis; D: distant metastasis; E: TNM stage; F: overall survival: there was a significant link between IL20R1/IL20R2/IL22R/IL24 combined and the overall survival of the patients (p=0.032).*

none were significantly correlated with the overall survival of the patients (*IL20R1*, p=0.47; *IL20R2*, p=0.068; *IL22R1*, p=0.32, *IL24*, p=0.27). However, when the four molecules were collectively analyzed, there was a significant survival link and higher levels of all molecules were associated with better survival (p=0.032, by Kaplan–Meier method) (Figure 1F).

Expression of IL24 and its receptors in pancreatic cancer cell lines. The expression of *IL24* was below the detection limit in tumour tissue and two pancreatic cancer cell lines; PANC1 cell line presented positive expression of *IL24* at the mRNA level, but western blot for IL24 protein was negative for all the three cell lines. *IL20R2* also presented consistent transcript

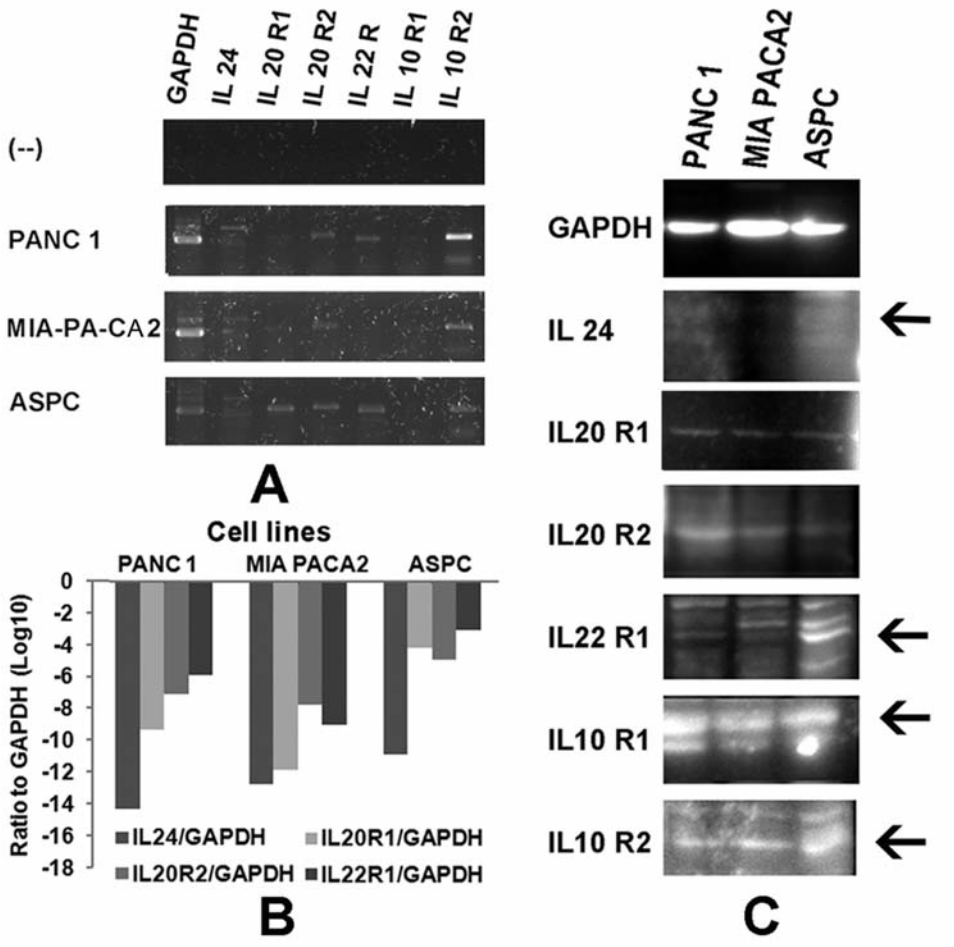

Figure 2. *Expression patterns of interleukin 24 (IL24) and interleukin 10 (IL10) family receptor subunits in pancreatic cancer cell lines. A: The pattern of IL24 receptor subunit expression by reverse transcription-polymerase chain reaction (RT-PCR) differed among the three pancreatic cancer cell lines. B: Patterns of IL24 receptor subunit expression detected by quantitative RT-PCR were consistent with those detected by RT-PCR. C: Confirmation of IL24 receptor subunit expression patterns using western blot. Expressions of most subunits were consistent at both mRNA and protein levels except for IL20R1, for which the protein expression level was higher than that of transcript expression.*

expression in frozen tumour tissues and cell lines. *IL20R2* transcript expression was between that of the other IL24 receptor subunits; *IL20R2* also exhibited consistent expression at the mRNA and protein levels in the three pancreatic cancer cell lines. While the transcript expression of *IL20R1* and *IL22R1* in cancer cell lines were reversed to that of tumour tissues. In the clinical cohort, *IL20R1* transcript expression was much higher than that of *IL22R1* and *IL22R1* expression was lower than that of *IL20R2*, but in cancer cell lines, *IL22R1* transcript expression was much higher than of *IL20R1*, and *IL20R1* expression was similar to that of *IL20R2*. Western blot revealed that IL22R1 expression was consistent at both mRNA and protein levels, and was negative in MIA PACA2 cell line. *IL20R1* expression was only positive in the ASPC cell line at the mRNA level, but was positive in all three cell lines at the protein level, with the ASPC cell line having higher expression level than PANC1 and MIA PACA2 (Figure 2).

Impact of recombinant IL24 protein on in vitro cell growth. Treatment under different recombinant IL24 protein concentrations for 3 days inhibited growth of PANC1, MIA PACA2 and ASPC cells, and the inhibitory effect was enhanced with increasing concentration (Figure 3).

Impact of recombinant IL24 protein on adhesion and migration. We further examined the effect of recombinant IL24 protein on pancreatic cancer cell adhesion. A general adhesion assay on Matrigel revealed no obvious difference among the different concentrations, neither did the ECIS-based attachment assays.

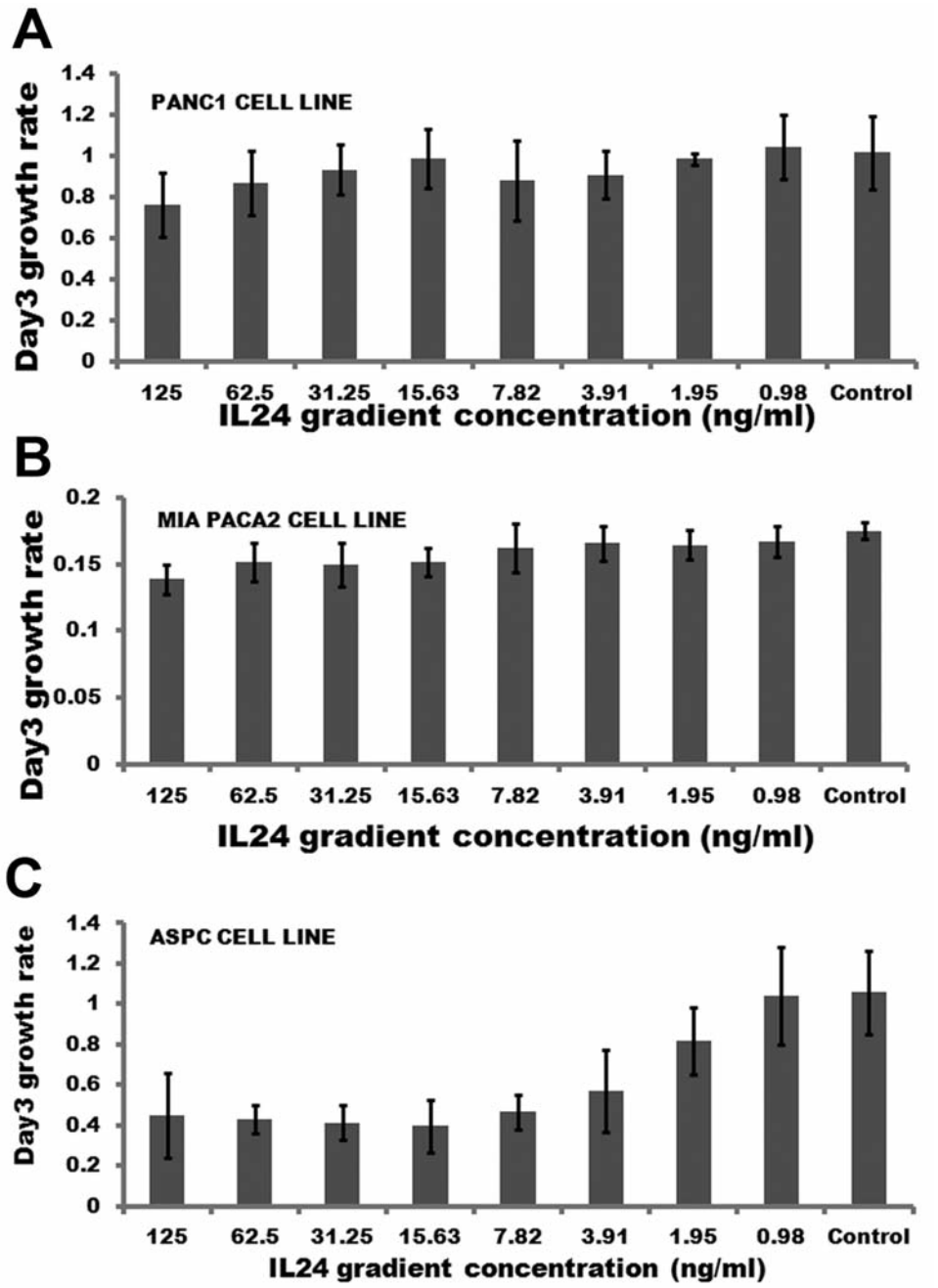

Figure 3. *Effect of recombinant interleukin 24 (IL24) protein on cell growth. A: PANC1: recombinant IL24 protein inhibited cell growth. The difference was significant at 125 ng/ml. B: MIA PACA2: recombinant IL24 protein also inhibited cell growth; the significant inhibitory effect was observed from 15.63 ng/ml. C: ASPC: Growth was dramatically inhibited by recombinant IL24 protein; the inhibitory effect was observable from 3.91 ng/ml onwards.*

The classical wounding assay was employed to investigate the effect of recombinant IL24 protein at 31 ng/ml on migration. Recombinant IL24 protein significantly inhibited the migration of PANC1, MIA PACA2 and ASPC cell lines. Further investigation with ECIS revealed that migration was inhibited at different concentrations (from 16 ng/ml to 62 ng/ml), and the inhibitory effect increased from lower to higher concentration in both PANC1 and ASPC cell lines, but not in MIA PACA2 cell line (Figure 4). Finally, the presence of IL24 recombinant protein had no effect on cancer cell adhesion and invasion (data not shown).

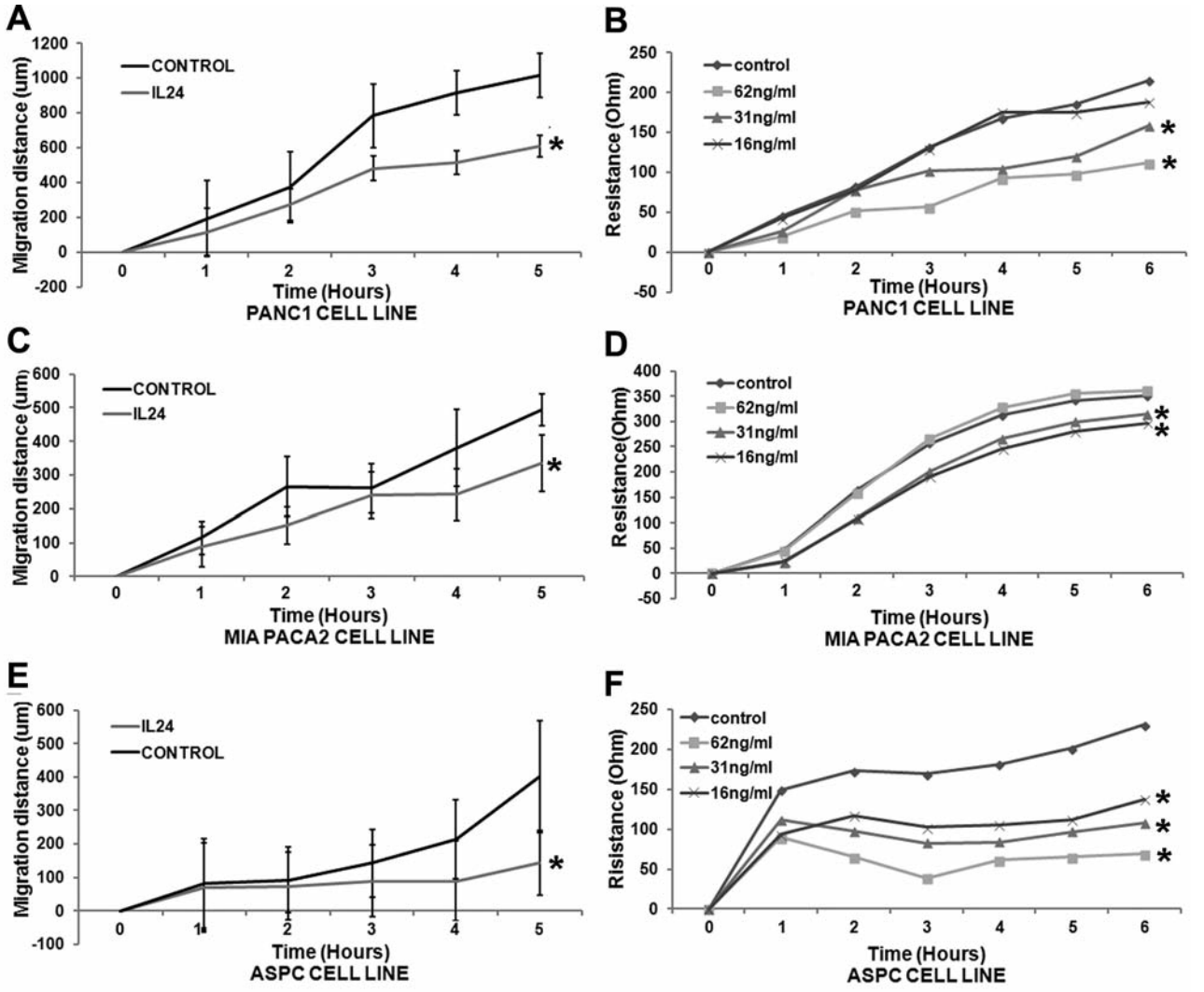

Figure 4. *Recombinant interleukin 24 (IL24) protein has an impact on pancreatic cancer cell migration. A: IL24 inhibited PANC1 cell migration at 31 ng/ml; B: IL24 inhibitory effect on PANC1 cells was enhanced with increasing concentration; C: MIA PACA2 cell migration was inhibited by IL24 at 31 ng/ml; D: the inhibitory effect of IL24 on MIA PACA2 cell migration decreased with increasing concentration, with the inhibitory effect on cellular migration being completely diminished at the concentration of 62 ng/ml; E: IL24 inhibited ASPC cell migration at 31 ng/ml; F: IL24 inhibitory effect on ASPC cells increased with increasing concentration.*

Discussion

Pancreatic cancer is a highly fatal disease characterized by extensive tumour invasion, early metastasis and marked cachexia. The 1- and 5-year survival rates are 25% and 5%, respectively. The only curative treatment of pancreatic cancer is radical surgery. Improvement of surgery and perioperative chemotherapy or chemoradiotherapy could increase the 5-year survival up to 20% in patients undergoing pancreaticoduodenectomy. While only patients with stage I disease and some with stage II are considered candidates for curative resection, this group only constitutes 10-20% of all patients with pancreatic cancer. For those with unresectable, locally advanced pancreatic cancer and those with metastatic disease, gemcitabine-based chemotherapy and chemoradio-therapy can improve the prognosis, yet the overall outcome is still far from satisfactory. The addition of elotinib (an oral tyrosine kinase inhibitor of the human epidermal growth factor receptor) to gemcitabine has proved to be able to prolong survival (8, 9); this fact reminds us of the importance of molecular targeted therapies.

IL24 is also a potential candidate for tumour molecular targeted therapy, its proapoptotic role has been confirmed and reported in many kinds of malignant tumour (13, 15, 16, 27,

28). Phase I clinical studies using nonreplicating adenoviral vector expressing *IL24* (INGN-24) were carried out in sequential cohorts of patients, and the clinical effect observed was similar to IL24 effects on cancer cell lines and xenograft mouse models (13, 17, 29). In addition, IL24 was reported to be able to radiosensitize lung tumour (16, 27, 30, 31).

IL24 receptors mediate IL24 molecular targeted therapy, however, the correlation of IL24 receptor expression with the effect of IL24 therapy remains uninvestigated. In our study, we aimed to investigate the role of IL24 receptor subunits in a pancreatic cancer cohort, and further explore the advantage of IL24 receptor subunit detection before IL24 molecular therapy.

IL24 exerts its biological effects through binding to IL20R1–IL20R2 and IL22R1– IL20R2 complexes. Among the three subunits of IL24 receptor; IL20R2 is the molecule common to the two complexes. The IL20R1 and IL22R1 expression levels determine the quantity of these two receptor complexes. We found that in pancreatic cancer cell lines, the expression of the three subunits was consistent at mRNA and protein levels, except for IL20R1; *IL20R1* were negative after 30 cycles of RT-PCR in PANC1 and MIA PACA2 cell lines, but it was detectable in western blot, thus we assume that *IL20R1* translational product was at a higher level compared to the transcriptional product. In tumour tissues, *IL20R1* transcript expression was much higher than that of *IL20R2*, and *IL22R1* expression was the lowest, which indicates that the IL20R1–IL20R2 complex was predominant in tumour tissues. However, in pancreatic cancer cell lines, *IL22R1* transcript expression was the highest, with *IL20R1* expression being a little lower than that for *IL20R2*, indicating that the IL22R1–IL20R2 complex was predominant in tumour cell lines, while in the IL22R-negative MIA PACA2 cell line, the IL20R1–IL20R2 complex was predominant.

According to the data of the clinical cohort, IL20R1 and IL20R2 expressions were directly related to tumour differentiation, but high IL22R1 expression corresponded to poor differentiation. As the expression of IL22R1 and IL20R2 were not consistent, we suppose that the reason for this may be that IL22R1 might also constitute the IL22R1–IL10R2 complex and act as the receptor for IL22.

In relation to tumour stage, IL20R1 and IL20R2 expressions were inversely related to tumour stage, but high IL22R1 transcript expression corresponded to higher T stage. In pancreatic cancer, the tumour stage is estimated according to tumour size, and the balance of tumour cell growth and death directly determine this size. The clinical cohort result indicates that the IL20R1–IL20R2 complex was involved in regulation of pancreatic cancer growth and apoptosis in the context of pancreatic cancer.

IL24 was reported to be able to promote apoptosis and inhibit growth through both complexes in various tumour cell lines. In our study, the three pancreatic cancer cell lines all exhibited growth inhibition after 3 days of treatment with recombinant IL24 protein. In PANC1 and ASPC cell lines, *IL22R1* transcript expression was much higher than that in tumour tissues, therefore both IL20R1–IL20R2 and IL22R1–IL20R2 complexes likely exist. The MIA PACA2 cell line on the contrary was negative for *IL22R1*, hence only the IL20R1–IL20R2 complex can exist, but growth inhibition was not any less. Based on the data above, we assume that the IL20R1–IL20R2 complex mediates the growth inhibition induced by IL24, but the involvement of IL22R1–IL20R2 complex in regulation of growth is still not verified.

The correlation of the expression of the three subunits and tumour N and M stage was also investigated in our study. The high levels of IL20R1 and IL20R2 corresponded to advanced N stage and metastasis, indicating that the IL20R1–IL20R2 complex may promote migration and metastasis in pancreatic cancer. The classical scratch assay revealed that tumour cell migration in three cell lines could be inhibited by recombinant IL24 protein at 31 ng/ml. The ECIS assay indicated that the inhibition by IL24 of migration was directly proportional to the IL24 concentration (from 31 to 125 ng/ml) in both PANC1 and ASPC cell lines. In contrast, in MIA PACA2 cell line, the inhibitory effect was inversely related to IL24 concentration, the migration under an IL24 concentration of 62.5 ng/ml was faster than that of control cells. This points to the IL22R1–IL20R2 complex being predominant in PANC1 and ASPC cell lines. The inhibition of migration may be *via* a combination of IL24 and IL22R1–IL20R2 complex, while IL22R1 was absent from MIA PACA2 cells. IL24 only binds to IL20R1–IL20R2 complex, so the two complexes may mediate different effects of recombinant IL24 protein on pancreatic cancer cell migration. In PANC1 and ASPC cell lines, it may be that the IL22R1–IL20R2 complex was predominant, therefore, only migration inhibition was observed, however, in the pancreatic cancer cohort, the IL20R1–IL20R2 complex was predominant, either high dosage of recombinant IL24 protein or high IL20R1–IL20R2 expression in tumour tissue could promote tumour cell migration.

IL24 overexpression promotes apoptosis in various tumour cell lines; the mechanism involved includes promotion of p38 mitogen activated protein kinase, protein kinase R, and c-Jun N-terminal kinase pathways and inhibition of wingless-type MMTV integration site family, and phosphatidylinositol 3-kinase pathways (12, 14, 16, 28, 30-33). In pancreatic cancer, nullifying Kirsten rat sarcoma viral oncogene homolog expression or inducing reactive oxygen species eliminated IL24 transcriptional blocking, and restored the proapoptotic effect of IL24 overexpression (15, 34-37).

In our study, recombinant IL24 protein also directly inhibited growth of pancreatic cancer cell lines, this result was consistent with the 'by-stander effect' reported by other studies (13, 38).

Although IL22R1 may mediate IL24 inhibition of pancreatic cancer cell migration, it is expressed at a low level in pancreatic tumour tissue; hence the IL22R1–IL20R2 complex may not be useful as a molecular marker for IL24 molecular therapy. However, *IL20R1* transcript expression was significantly related to T stage, despite the correlation of high IL20R1 expression with advanced N stage, M stage and the fact that IL20R1-IL20R2 complex may promote migration with response to high IL24 concentration, higher IL20R1 expression correspond to lower TNM stage and better survival. The interaction between IL24 and its receptors and their role in pancreatic cancer appears complex and may influence numerous stages of disease progression. We come to the conclusion that IL20R1 is a potential molecular marker for IL24 molecular therapy in pancreatic cancer.

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