Silencing of the ARP2/3 Complex Disturbs Pancreatic Cancer Cell Migration

HANNA E. RAUHALA[1,2,3], SUSANNA TEPPO[1,2,3], SANNA NIEMELÄ[1,2,3] and ANNE KALLIONIEMI[1,2,3]

[1]*Institute of Biomedical Technology, University of Tampere, Tampere, Finland;*
[2]*BioMediTech, Tampere, Finland;*
[3]*Fimlab Laboratories, Tampere, Finland*

Abstract. *Background: Actin-related protein 2/3 (ARP2/3) complex is an actin nucleator responsible for actin cytoskeleton branching which is essential for efficient cell migration. Materials and Methods: The expression of the seven ARP2/3 complex subunits was assessed in pancreatic cancer cell lines and in normal pancreatic samples by quantitative RT-PCR. siRNA-mediated silencing was used to study the contribution of each ARP2/3 complex member to pancreatic cancer cell migration. Results: ARPC3 and ARPC4 were the most highly expressed complex members, while ARPC1B and ARPC2 were expressed at low levels. Silencing of the ARP2/3 complex subunits typically resulted in reduced cell migration capacity. In particular, silencing of ARPC4 significantly reduced cell migration in all studied cell lines, with a major impact on Hs700T and HPAFII migration (50% and 68% decrease, p<0.001). Conclusion: We offer comprehensive expression data on the ARP2/3 complex members for pancreatic cancer and normal pancreas. In addition, we show cell line-specific differences in ARP2/3 complex subunit dependency on cell migratory potential, and suggest ARPC4 to be one of the key members of the ARP2/3 complex in pancreatic cancer.*

Cellular movement is essential to several normal biological processes, such as the activities of the immune system and tissue repair and regeneration, while aberrantly activated cell migration is involved in many diseases. For example in cancer, acquired cell migration ultimately leads to lethal metastatic disease. Indeed, the ability to form metastases is defined as one of the hallmarks of a cancer cell (1). The metastatic process, encompassing dissemination from the primary site, transport *via* the blood stream or lymphatic system to a new location, invasion and colonization into distant tissue, involves key cellular changes, such as changes in cell-to-cell adhesion, induction of epithelial–mesenchymal transition (EMT) and altered tumour-to-stroma crosstalk (2-4). In all these events associated with cell migration, actin cytoskeleton remodelling is involved to achieve the proper cellular outcome.

The actin cytoskeleton is composed of monomeric globular actin (G-actin) that self-assembles into filamentous F-actin upon hydrolysis of ATP (5). Major cellular functions of actin include mechanical support for the cell, cellular vesicle trafficking, muscle contraction, cell division, cell motility and cell adhesion. Many of these functions, including cell motility and cell adhesion, involve contact with the plasma membrane, thus extending the actin network outside of the cell, in order to enable the actin cytoskeleton to respond to extracellular signals, such as chemoattractants (6, 7). In cell migration, membrane protrusions are created at the leading edge of the cell, where actin cytoskeleton re-organization generates the motile force. Depending on the morphological, structural and functional characteristics of these protrusions, they are termed invadopodia, filopodia and lamellipodia (8). Invadopodia have the capacity to degrade extracellular matrix (ECM) and are particularly linked to cell invasion (9). Filopodia are long thin membrane protrusions where parallel unbranched actin filaments are tightly bundled (10). Filopodia are thought to act as pioneers of the leading edge by probing the environment for cues (11). Lamellipodia, in turn, are flat sheet-like membrane protrusions with branched actin structures (8, 12). A key component of these lamellipodial actin structures is the actin-related protein 2/3 (ARP2/3) complex that functions as an actin nucleator and mediates actin filament branching (13). These actin nucleation factors are important accelerators of actin polymerization by introducing the monomeric actin units to the existing actin filament ends or branch sites (14). The ARP2/3 complex is an evolutionally well-conserved

Correspondence to: Professor Anne Kallioniemi, Institute of Biomedical Technology, FIN_33014 University of Tampere, Finland. E-mail: anne.kallioniemi@uta.fi

Key Words: ARP2/3 complex, pancreatic cancer, cell migration.

seven-subunit protein complex consisting of two proteins structurally similar to actin, namely the actin-related proteins 2 and 3 (ACTR2 and ACTR3), and of additional five actin-related protein 2/3 complex subunits (ARPC1, ARPC2, ARPC3, ARPC4 and ARPC5) (15, 16). ARPC1 has two isoforms in humans, ARPC1A and ARPC1B. At the actin branch site, ACTR2 and ACTR3 are in contact with the pointed end of the novel daughter filament, while ARPC2 and ARPC4 connect the complex to the mother filament. The exact functions of the β-propeller protein ARPC1, and ARPC3 and ARPC5 situated at the edge of the complex, remain unknown (17).

While several knockdown studies have demonstrated how the ARP2/3 complex disruption in mouse embryonic fibroblasts leads to inhibition of lamellipodia formation, thus impairing cell migration (18-20), there are also data showing no evident effect of ARP2/3 depletion on lamellipodia structure (21). Recently, two studies demonstrated how ARP2/3 complex is indeed critical for lamellipodia formation, and even though disruption of this complex did not entirely abolish cell migration, it did influence the directionality of cell movement, making mouse embryonic fibroblasts unable to sense changes in the ECM and move along these gradients in a coherent manner (22, 23). Instead of lamellipodia, these ARP2/3 complex-depleted cells formed filopodial structures to support their migration. Further support for the importance of ARP2/3 complex in lamellipodia formation and migration came from a study showing that the correct localization of *ACTR2* mRNA at the protrusions was crucial for the proper directionality of cell migration (24).

We previously identified *ARPC1A* and *ARPC1B* as putative target genes for the 7q22 amplification in pancreatic cancer and showed that the migration potential of cells harbouring this amplification can be reduced using small interfering RNA (siRNA)-mediated silencing of *ARPC1A* and *ARPC1B* (25). Here, we undertook a systematic study on the role of this complex in pancreatic cancer by evaluating the expression levels of all ARP2/3 complex subunits and the effects of silencing each complex member on pancreatic cancer cell migration.

Materials and Methods

Cell lines and normal samples. AsPC-1, BxPC-3, Capan-1, Capan-2, CFPAC-1, HPAC, HPAF-II, Hs700T Hc766T, MiaPaCa-2, PANC-1, SU.86.86 and SW1990 pancreatic cancer cell lines and human telomerase reverse transcriptase (hTERT)-immortalized non-malignant human pancreatic epithelial cells (hTERT-HPNE) were obtained from the American Type Culture Collection (ATCC, Manassas, CA, USA). DAN-G, Hup-T3 and Hup-T4 pancreatic cancer cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (Brunswick, Germany). All cells were grown under the conditions recommended by the provider. Four normal human pancreatic total RNAs were obtained from Ambion (Life Technologies, Grand Island, NY, USA), Clontech Laboratories, Inc. (Mountain View, CA, USA) and BioChain Institute, Inc. (Newark, CA, USA).

Gene expression analysis by real-time quantitative RT-PCR. For the expression analysis, total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). RNAs were reverse transcribed using SuperScript III® First-Strand Synthesis System for RT-PCR (Life Technologies). Real-time quantitative PCR was then performed with Universal ProbeLibrary (UPL) system (Roche, Mannheim, Germany) using a Light Cycler 2.0 apparatus (Roche). Briefly, amplicons for each gene were designed using Universal ProbeLibrary Assay Design Center online. Selected primers were ordered from Proligo (Sigma-Aldrich, St. Louis, MO, USA) and the UPL probes from Roche. The real-time qPCR was run according to the manufacturer's instructions. For normalization, the expression of glyceraldehyde-3-phosphate dehydrogenase (*GAPD*) gene was measured using Universal ProbeLibrary Human GAPD Gene Assay (Roche). For the cell lines and normal pancreatic samples, the expression values were calculated in relation to the median value of all the genes across the samples to allow for easier comparison between the genes and the samples.

siRNA transfections. ON-TARGET plus SMART siRNA pools for each gene were purchased from Thermo-Fisher Scientific (Waltham, MA, USA). Each pool was a mixture of four individual gene-specific siRNAs. siRNAs were transfected at final concentration of 8 μM using INTERFERin® transfection reagent (Polyplus Transfection, IllKirch, France). Control siRNA targeting the firefly luciferase gene (*LUC*) was obtained from Sigma-Aldrich. Silencing efficiency was analyzed 48 h post-transfection using qRT-PCR. Silencing efficiency ranged from 62 to 96% (Table I).

Cell migration assay. Migration was studied using cell culture inserts with 8-μm pores, according to the manufacturer's instructions (BD Falcon, Franklin Lakes, NJ, USA). Briefly, 100,000 cells were seeded in 1% fetal bovine serum (FBS) containing medium into migration chambers 48 h post-transfection. Cells were allowed to migrate through the pores into the lower well containing 10% FBS medium for approximately 22 h, after which the migrated cells were methanol-fixed and stained with 1% toluidine blue in 1% borax. Membranes were mounted on microscopic slides and images were captured using AperioScanScope®XT (software version 9; Aperio, Vista, CA, USA). Cell migration was assessed using the ImageJ software (http://rsbweb.nih.gov/ij/) with total count of migrated cells per membrane as the analysis output. Each assay was performed in 4-6 replicates and repeated at least twice.

Statistical analysis. The Mann–Whitney *U*-test was performed to compare the median values of control and test groups.

Results

ARP2/3 complex expression in pancreatic cancer cell lines. The expression of the ARP2/3 complex subunits was analyzed with qRT-PCR in 16 pancreatic cancer cell lines, hTERT-HPNE cells and in four normal pancreatic tissue samples (Figure 1, Table II). Among the most highly

Table I. *Silencing efficiencies of the actin-related protein 2/3 (ARP2/3) complex subunits in the AsPC-1, Hs700T and HPAFII cell lines. Silencing efficiencies for each siRNA in each cell line are represented as the percentage of down-regulation of expression, in comparison to the expression of siLUC-transfected cells 48 h post-transfection.*

	Silencing efficiency (range)
AsPC-1	
siARPC1A	78-90%
siARPC1B	65-90%
siARPC2	89-92%
siARPC3	77-91%
siARPC4	74-91%
siARPC5	72-77%
siACTR2	63-83%
siACTR3	62-90%
Hs700T	
siARPC1A	89-95%
siARPC1B	83-91%
siARPC4	94-96%
HPAFII	
siARPC1A	71-81%
siARPC1B	69-88%
siARPC4	87-89%

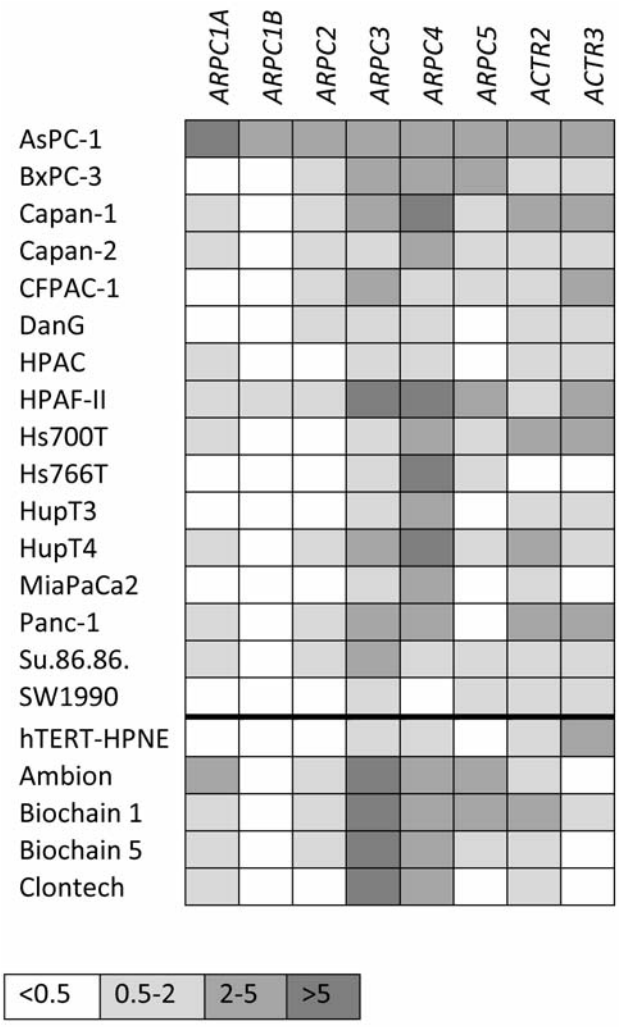

Figure 1. *A schematic representation of the expression levels of the actin-related protein 2/3 (ARP2/3) complex subunits in 16 pancreatic cancer cell lines, hTERT-HPNE cell line and four normal pancreatic samples. Expression values of each gene were normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPD) housekeeping gene, and are shown relative to the median value of all genes across all samples.*

expressed ARP2/3 complex subunits was *ARPC1A* in AsPC-1 cells, *i.e.* the cell line that has high-level amplification of the *ARPC1A* and *ARPC1B* genes (25). For *ARPC1B*, low expression levels as compared to the other complex members were observed across the sample set, except for AsPC-1 cells. In a similar fashion, *ARPC2* expression levels were low and uniform across all the samples. For most of the samples, *ARPC3* and/or *ARPC4* were the ARP2/3 complex members with highest expression level. *ARPC5*, *ACTR2* and *ACTR3* were expressed at rather uniform levels across the samples. In general, it appeared that the expression levels of the ARP2/3 complex members were relatively uniform within any particular cell line. For example, AsPC-1 and HPAFII cells had high expression, whereas MiaPaCa2 and SW1990 cell lines had low expression of most of the subunits. Interestingly in the hTERT-HPNE cells, the expression levels of all ARP2/3 complex members were rather low, while much more variation was seen among normal pancreas samples (Figure 1).

Effect of ARP2/3 complex silencing on cell migration. We have previously shown that silencing of *ARPC1A* and *ARPC1B* in the AsPC-1 cell line led to reduced cellular migration (25). We wanted to see if the same holds true for the other cell lines. To this end, we used siRNAs to specifically silence *ARPC1A* and *ARPC1B* expression in the Hs700T and HPAFII cell lines, as well as in the AsPC-1 cell line. In AsPC-1 cells, we were unable to replicate our previous results, as we did not observe any significant changes in the migration ability of these cells after silencing of *ARPC1A* or *ARPC1B* (Figure 2). One explanation for these rather surprising results could be the properties of the migration chambers used, as the overall migration ability of the AsPC-1 cells was markedly lower in our earlier studies than in the experiments performed here. In Hs700T cells, silencing of *ARPC1A* significantly reduced migration to 51%, as compared to si*LUC*-transfected cells (p=0.0029, Figure 2), whereas silencing of *ARPC1B* led to a 28%

Table II. *Glyceraldehyde-3-phosphate dehydrogenase (GAPD) normalized actin-related protein 2/3 (ARP2/3) complex subunit expression values in 16 pancreatic cancer cell lines, hTERT-HPNE cell line and four normal pancreatic samples.*

	ARPC1A	*ARPC1B*	*ARPC2*	*ARPC3*	*ARPC4*	*ARPC5*	*ACTR2*	*ACTR3*
AsPC-1	9.95	3.27	2.37	4.42	3.71	2.57	4.44	3.73
BxPC-3	0.38	0.13	1.18	2.26	3.81	2.03	0.71	1.64
Capan-1	0.99	0.45	1.52	3.84	7.20	0.66	3.11	2.26
Capan-2	0.87	0.21	0.58	1.47	2.03	1.89	1.85	1.51
CFPAC-1	0.45	0.24	0.75	4.45	0.87	1.41	1.41	2.11
DanG	0.13	0.06	1.62	1.05	0.99	0.32	0.93	0.65
HPAC	0.54	0.09	0.19	0.52	1.66	0.26	0.59	0.62
HPAFII	1.40	0.65	1.10	10.56	7.25	2.53	1.93	3.00
Hs700T	0.67	0.14	0.32	0.71	4.10	1.52	2.75	2.56
Hs766T	0.29	0.07	0.49	0.76	15.42	1.63	0.31	0.29
HupT3	0.25	0.04	0.28	1.97	3.34	0.45	1.99	1.85
HupT4	1.00	0.18	0.98	3.07	10.41	1.11	3.57	0.98
MiaPaCa2	0.45	0.07	0.25	1.21	4.48	0.24	1.15	0.26
PANC-1	0.91	0.14	0.67	3.67	2.03	0.39	4.92	3.60
Su.86.86	0.53	0.42	0.80	2.33	0.81	1.63	1.73	1.26
SW1990	0.25	0.12	0.48	1.15	0.32	0.84	1.00	0.66
hTERT-HPNE	0.04	0.01	0.41	0.64	0.83	0.20	1.07	2.45
Ambion	2.12	0.27	0.54	6.42	3.36	4.18	1.15	0.36
Biochain 1	1.86	0.14	1.30	15.75	4.96	4.04	2.02	0.88
Biochain 5	1.27	0.50	0.95	7.79	3.50	1.46	1.66	0.28
Clontech	0.68	0.13	0.47	5.31	2.46	0.29	0.93	0.46

increase in cell migration (p=0.0218, Figure 2). Silencing of *ARPC1A* and *ARPC1B* in HPAFII cells caused a statistically significant increase in the migration (30%, p=0.0435 and 46%, p=0.0015 increase, respectively; Figure 2). We then confirmed that the silencing of *ARPC1A* did not have any significant effect on *ARPC1B* expression and *vice versa* (data not shown), supporting the specificity of the siRNAs when silencing two relatively homologous genes. Additionally, it shows that the expression of *ARPC1A* and *ARPC1B* does not seem to be regulated in a complementary manner, where the down-regulation of one would lead to up-regulation of the other, thus alleviating the total effect on the function of the ARP2/3 complex. Taken together, interfering with *ARPC1A/B* function leads to cell line-specific responses in cell migration, with differences in the magnitude and direction of the change in the migration potential.

Next, we studied the effects of silencing of the other ARP2/3 complex subunits in the AsPC-1 cell line, as this cell line expressed all the complex members at a rather high level (Figure 1). Decreased expression of *ARPC2*, *ARPC3* and *ARPC4* significantly reduced the migratory capacity of the cells as compared to LUC control (35%, p=0.0002; 31%, p=0.0022; and 24%, p=0.0066, respectively; Figure 3), while silencing of the *ARPC5* subunit did not have any major effect on cell migration (p=0.1489, Figure 3). Silencing of the two main components of the complex, *ACTR2* and *ACTR3*, reduced migration by 35% and 24% (p=0.0017 and p=0.0196, respectively; Figure 3). These results imply that while disturbing the balance of the ARP2/3 complex clearly affects the migration potential of AsPC-1 cells, it does not fully abolish their migratory capacity. Since *ARPC4* was highly expressed in most of the pancreatic cancer cell lines and, to our knowledge, no previous data existed on the role of *ARPC4* in the regulation of migration in cancer cells, we chose to evaluate the effects of its silencing in the Hs700T and HPAFII cell lines. In both cell lines, decreased *ARPC4* expression led to significant reduction in cell migration as compared to *LUC* control (50%, p=0.0002; and 68%, p=0.0006, respectively; Figure 4). Again, the effect of silencing of the ARP2/3 complex subunit led to differing outcomes in different cell lines. While the depletion of *ARPC4* affected migration in AsPC-1 cells only moderately, the effect was substantial in Hs700T and HPAFII.

Discussion

We performed a comprehensive screening of ARP2/3 complex gene expression and the effects of silencing of the complex subunits on cell migration in pancreatic cancer. Pancreatic cancer cell lines and normal pancreas expressed the ARP2/3 complex members at differing levels, although for any particular sample, the complex subunits were expressed at relatively equal levels. In terms of different subunits, *ARPC1B* and *ARPC2* were typically expressed at low levels, whereas *ARPC3* and *ARPC4* were highly expressed. When comparing our qRT-PCR data to two

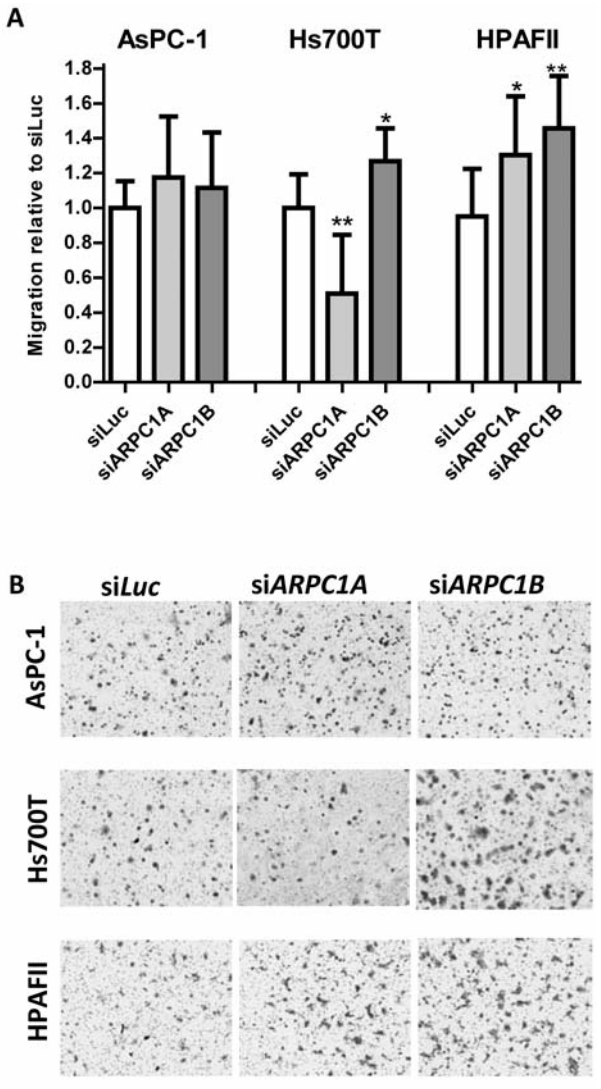

Figure 2. *Silencing of actin-related protein 2/3 complex, subunit 1A (ARPC1A) or ARPC1B in pancreatic cancer cell lines influences cell migration in a cell line-specific manner. A: AsPC-1, Hs700T and HPAFII cells were collected 48 h after transfection with the indicated siRNAs and subjected to a migration assay. The migration data are represented as the mean and SD relative to that of the luciferase-transfected (siLUC) control (*p<0.05, **p<0.01). B: Representative images of fixed and stained cells in the migration assays at ×10 magnification.*

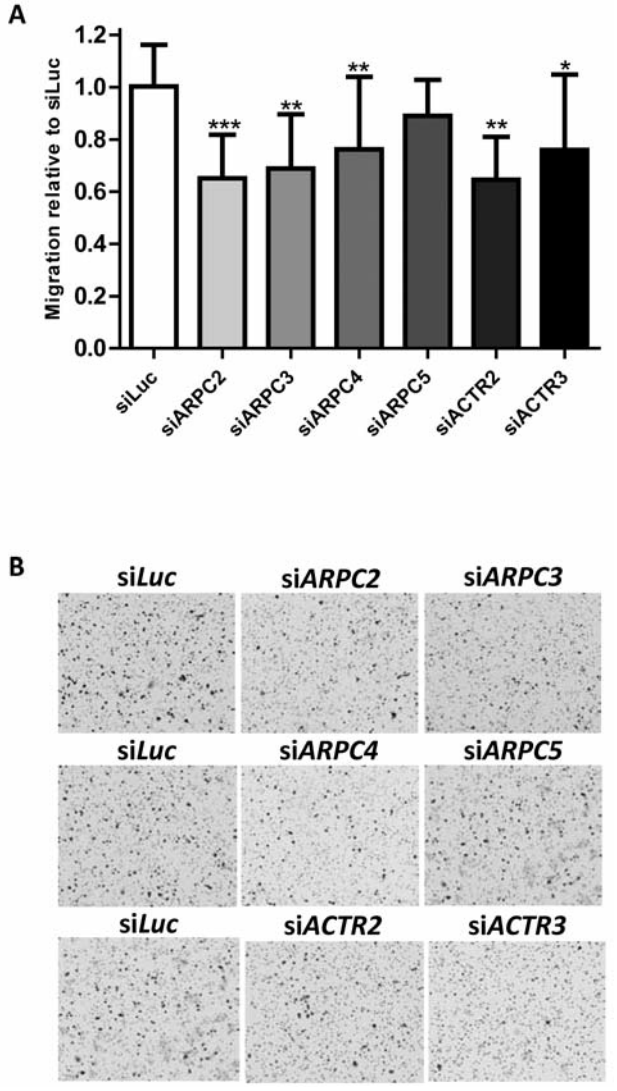

Figure 3. *Silencing of actin related protein 2/3 complex, subunit 2 (ARPC2), ARPC3, ARPC4, ARPC5, actin-related protein 2 homolog (ACTR2) or ACTR3 in the AsPC-1 cell line reduced cell migration at differing levels. A: AsPC-1 cells were collected 48 h after transfection with the indicated siRNAs and subjected to a migration assay. The migration data are represented as the mean and SD relative to that of the luciferase-transfected (siLUC) control (*p<0.05, **p<0.01, ***p<0.001). B: Representative images of fixed and stained cells in the migration assays at ×10 magnification.*

published pancreatic cancer cell line microarray data sets [Cancer Cell Line Encyclopedia (CCLE) in 26; 27] most of the overlapping cell lines showed concordant expression levels. *ARPC1A*, *ARPC1B* and *ARPC3* were the genes most consistently expressed between the different data sets (*ARPC3* data are lacking from CCLE). Some variation between the data sets was seen *e.g.* for *ARPC4*, whose expression levels were approximately 10-fold lower than those of the other ARP2/3 complex members in the CCLE data, whereas in our data and in the data by Shain and colleagues (27), *ARPC4* was expressed at higher levels than the other ARP2/3 complex members. We also saw consistently low expression of *ARPC2*, but it was the highest expressed ARP2/3 complex subunit in the CCLE data. Similarly, the IST database (ist.genesapiens.org), containing expression data from normal and tumour samples, had

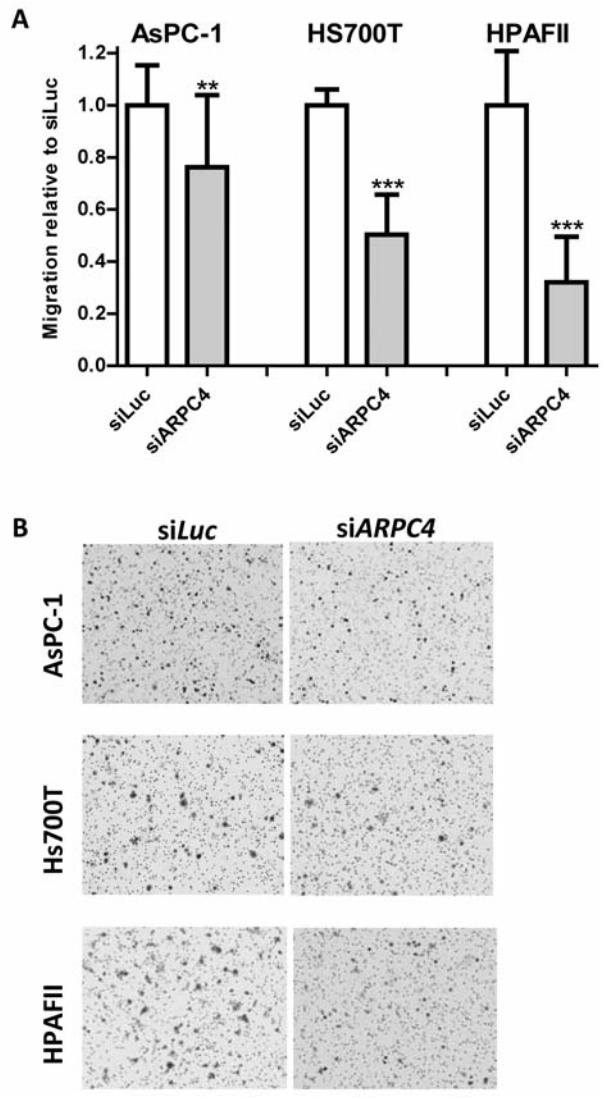

Figure 4. *Silencing of actin related protein 2/3 complex, subunit 4, 20kDa (ARPC4) in pancreatic cancer cell lines caused a dramatic decrease in cell migration. A: Hs700T and HPAFII cells were collected 48 h after transfection with the indicated siRNAs and subjected to a migration assay. The migration data are represented as the mean ± SD relative to that of the luciferase-transfected (siLUC) control (**p<0.01, ***p<0.001). Data for AsPC-1 cells from Figure 3 is shown here for the purpose of comparison. B: Representative images of fixed and stained cells in the migration assays at ×10 magnification.*

ARPC2 as the highest expressed ARP2/3 complex member in pancreatic cancer (*ARPC4* data are lacking from the IST database). Overall, the concordance between different data sets was good, and the few discrepancies most likely arise from the different platforms (Affymetrix in CCLE and IST database, Agilent in Shain *et al*. and qRT-PCR in our data) and the different analytical methods used.

Silencing of almost any of the ARP2/3 complex subunits resulted in a significant decrease in the cell migratory capacity. Indeed, *ARPC2*, *ARPC3*, *ARPC4*, *ACTR2* or *ACTR3* depletion led to reduced migration of AsPC-1 cells. The most prominent effect on cell migration was seen with silencing of *ARPC4*. In all cell lines used, *ARPC4* silencing reduced migration, most dramatically in Hs700T and HPAFII cells, where the migration potential was reduced to half and to one-third, respectively. To our knowledge, this is the first time that *ARPC4* has been shown to influence cell migration. Most diverse effects were seen with *ARPC1A*, silencing of which caused cell line-specific responses, including reduced migration (Hs700T cells), no effect (AsPC-1 cells) and increased migration (HPAFII cells). Intriguingly, silencing of *ARPC1B* led to increased migration in both Hs700T and HPAFII cells, while no effect was seen on AsPC-1 cell migration. However, since the endogenous *ARPC1B* expression levels were already low, it is difficult to assess the importance of this result. Interestingly, *ARPC5* depletion did not affect the migration of AsPC-1 cells. The silencing efficiency of *siARPC5* was slightly lower than that of the other ARP2/3 complex members, but is unlikely this explains these results. In contrast, a previous study showed that silencing of *ARPC5* by miR-133 resulted in reduced migration of head and neck carcinoma cells (28). Similarly to our data, silencing of *ARPC2* led to significant reduction in invasion in breast cancer cells (29). Taken together, these results highlight the importance of the ARP2/3 complex in the migration and invasion of multiple cancer cell types. As might be expected, cells typically respond to ARP2/3 complex interference by reduced cell migratory capacity, although cell type-specific effects may exist. Nevertheless, some level of ARP2/3 complex function can be maintained even when one of the complex subunits is depleted.

Apart from these reports showing changes in cell migration and invasion in cancer when interfering with the ARP2/3 complex subunits, there are studies demonstrating aberrant expression of the complex members in clinical tumour samples. For example, overexpression of ARPC2 assessed by immunohistochemistry was included in a multimarker diagnostic assay that was able to correctly distinguish melanomas from benign and atypical nevi (30). Moreover, increased expression of ACTR2 and ACTR3 in colon cancer cells, as well as in the surrounding stromal cells, was shown to correlate with the malignancy stage of the tumour (31). In colon, breast and lung cancer, high ACTR2 expression co-localizing with the nucleation promoting factor WAS protein family, member 2 (WAVE2) has been shown to correlate with poor clinical outcome (32-34). Quite opposite to these findings, Kaneda and colleagues reported decreased expression of all of the ARP2/3 complex genes in gastric cancer when compared to normal adjacent tissue (35).

Our present study provides the expression patterns of the ARP2/3 complex members in a large set of pancreatic cancer cell lines. It is also the first, as far as we know, to comprehensively silence all the ARP2/3 complex members one by one and to study the effects on cell migration in pancreatic cancer. While ARP2/3 complex silencing did, in most cases, reduce cellular migration, it did not fully abolish it. This could reflect a change in the migration style, *i.e.* from chemotaxis-driven directional migration to more random directionally compromised migration, as has been suggested to happen in ARP2/3 complex-depleted mouse embryonic fibroblasts (22, 23). On the other hand, it could be argued that the residual expression of the siRNA-silenced ARP2/3 complex members is sufficient to maintain a functional ARP2/3 complex, thus leading only to reduced, not fully abolished, migration. Our results highlight the ARP2/3 complex subunit-dependent migration behaviour of pancreatic cancer cells. More studies are needed to fully understand the role and importance of each ARP2/3 complex member in the regulation of cell migration. This is of special interest in the context of cancer, where aberrations in ARP2/3 complex expression patterns are regularly found.

Acknowledgements

We thank Ms. Kati Rouhento for skilful assistance. This study was supported by grants from The Academy of Finland (Grant number 251066), The Sigrid Juselius Foundation, and the Competitive Research Funding of the Tampere University Hospital.

References

1 Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell *144*: 646-674, 2011.

2 Berx G and van Roy F: Involvement of members of the cadherin superfamily in cancer. Cold Spring Harb Perspect Biol *1*: a003129, 2009.

3 Joyce JA and Pollard JW: Microenvironmental regulation of metastasis. Nat Rev Cancer *9*: 239-252, 2009.

4 Polyak K and Weinberg RA: Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer *9*: 265-273, 2009.

5 Laki K, Bowen WJ and Clark A: The polymerization of proteins; adenosine triphosphate and the polymerization of actin. J Gen Physiol *33*: 437-443, 1950.

6 Kovacs EM, Goodwin M, Ali RG, Paterson AD and Yap AS: Cadherin-directed actin assembly: E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. Curr Biol *12*: 379-382, 2002.

7 Le Clainche C and Carlier MF: Regulation of actin assembly associated with protrusion and adhesion in cell migration. Physiol Rev *88*: 489-513, 2008.

8 Yamaguchi H and Condeelis J: Regulation of the actin cytoskeleton in cancer cell migration and invasion. Biochim Biophys Acta *1773*: 642-652, 2007.

9 Kelly T, Mueller SC, Yeh Y and Chen WT: Invadopodia promote proteolysis of a wide variety of extracellular matrix proteins. J Cell Physiol *158*: 299-308, 1994.

10 Nobes CD and Hall A: Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell *81*: 53-62, 1995.

11 Gupton SL and Gertler FB: Filopodia: The fingers that do the walking. Sci STKE 2007: re5, 2007.

12 Vinzenz M, Nemethova M, Schur F, Mueller J, Narita A, Urban E, Winkler C, Schmeiser C, Koestler SA, Rottner K, Resch GP, Maeda Y and Small JV: Actin branching in the initiation and maintenance of lamellipodia. J Cell Sci *125*: 2775-2785, 2012.

13 Firat-Karalar EN and Welch MD: New mechanisms and functions of actin nucleation. Curr Opin Cell Biol *23*: 4-13, 2011.

14 Le Clainche C, Pantaloni D and Carlier MF: ATP hydrolysis on actin-related protein 2/3 complex causes debranching of dendritic actin arrays. Proc Natl Acad Sci USA *100*: 6337-6342, 2003.

15 Welch MD, DePace AH, Verma S, Iwamatsu A and Mitchison TJ: The human Arp2/3 complex is composed of evolutionarily conserved subunits and is localized to cellular regions of dynamic actin filament assembly. J Cell Biol *138*: 375-384, 1997.

16 Robinson RC, Turbedsky K, Kaiser DA, Marchand JB, Higgs HN, Choe S and Pollard TD: Crystal structure of Arp2/3 complex. Science *294*: 1679-1684, 2001.

17 Goley ED and Welch MD: The ARP2/3 complex: an actin nucleator comes of age. Nat Rev Mol Cell Biol *7*: 713-726, 2006.

18 Bailly M, Ichetovkin I, Grant W, Zebda N, Machesky LM, Segall JE and Condeelis J: The F-actin side binding activity of the Arp2/3 complex is essential for actin nucleation and lamellipod extension. Curr Biol *11*: 620-625, 2001.

19 Rogers SL, Wiedemann U, Stuurman N and Vale RD: Molecular requirements for actin-based lamella formation in Drosophila S2 cells. J Cell Biol *162*: 1079-1088, 2003.

20 Steffen A, Faix J, Resch GP, Linkner J, Wehland J, Small JV, Rottner K and Stradal TE: Filopodia formation in the absence of functional WAVE- and Arp2/3-complexes. Mol Biol Cell *17*: 2581-2591, 2006.

21 Di Nardo A, Cicchetti G, Falet H, Hartwig JH, Stossel TP and Kwiatkowski DJ. Arp2/3 complex-deficient mouse fibroblasts are viable and have normal leading-edge actin structure and function. Proc Natl Acad Sci USA *102*: 16263-16268, 2005.

22 Suraneni P, Rubinstein B, Unruh JR, Durnin M, Hanein D and Li R: The Arp2/3 complex is required for lamellipodia extension and directional fibroblast cell migration. J Cell Biol *197*: 239-251, 2012.

23 Wu C, Asokan SB, Berginski ME, Haynes EM, Sharpless NE, Griffith JD, Gomez SM and Bear JE: Arp2/3 is critical for lamellipodia and response to extracellular matrix cues but is dispensable for chemotaxis. Cell *148*: 973-987, 2012.

24 Liao G, Simone B and Liu G: Mis-localization of Arp2 mRNA impairs persistence of directional cell migration. Exp Cell Res *317*: 812-822, 2011.

25 Laurila E, Savinainen K, Kuuselo R, Karhu R and Kallioniemi A: Characterization of the 7q21-q22 amplicon identifies ARPC1A, a subunit of the Arp2/3 complex, as a regulator of cell migration and invasion in pancreatic cancer. Genes Chromosomes Cancer *48*: 330-339, 2009.

26 Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, Wilson CJ, Lehár J, Kryukov GV, Sonkin D, Reddy A, Liu M, Murray L, Berger MF, Monahan JE, Morais P, Meltzer J, Korejwa A, Jané-Valbuena J, Mapa FA, Thibault J, Bric-Furlong E, Raman P, Shipway A, Engels IH, Cheng J, Yu GK, Yu J, Aspesi P Jr, de Silva M, Jagtap K, Jones MD, Wang L, Hatton C, Palescandolo E, Gupta S, Mahan S, Sougnez C, Onofrio RC, Liefeld T, MacConaill L, Winckler W, Reich M, Li N, Mesirov JP, Gabriel SB, Getz G, Ardlie K, Chan V, Myer VE, Weber BL, Porter J, Warmuth M, Finan P, Harris JL, Meyerson M, Golub TR, Morrissey MP, Sellers WR, Schlegel R and Garraway LA: The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature *483*: 603-607, 2012.

27 Shain AH, Giacomini CP, Matsukuma K, Karikari CA, Bashyam MD, Hidalgo M, Maitra A and Pollack JR: Convergent structural alterations define SWItch/Sucrose NonFermentable (SWI/SNF) chromatin remodeler as a central tumour suppressive complex in pancreatic cancer. Proc Natl Acad Sci USA *109*: E252-259, 2012.

28 Kinoshita T, Nohata N, Watanabe-Takano H, Yoshino H, Hidaka H, Fujimura L, Fuse M, Yamasaki T, Enokida H, Nakagawa M, Hanazawa T, Okamoto Y and Seki N: Actin-related protein 2/3 complex subunit 5 (ARPC5) contributes to cell migration and invasion and is directly regulated by tumour-suppressive microRNA-133a in head and neck squamous cell carcinoma. Int J Oncol *40*: 1770-1778, 2012.

29 Kim DH, Bae J, Lee JW, Kim SY, Kim YH, Bae JY, Yi JK, Yu MH, Noh DY and Lee C: Proteomic analysis of breast cancer tissue reveals upregulation of actin-remodeling proteins and its relevance to cancer invasiveness. Proteomics Clin Appl *3*: 30-40, 2009.

30 Kashani-Sabet M, Rangel J, Torabian S, Nosrati M, Simko J, Jablons DM, Moore DH, Haqq C, Miller JR 3rd and Sagebiel RW: A multi-marker assay to distinguish malignant melanomas from benign nevi. Proc Natl Acad Sci USA *106*: 6268-6272, 2009.

31 Otsubo T, Iwaya K, Mukai Y, Mizokami Y, Serizawa H, Matsuoka T and Mukai K: Involvement of Arp2/3 complex in the process of colorectal carcinogenesis. Mod Pathol *17*: 461-467, 2004.

32 Semba S, Iwaya K, Matsubayashi J, Serizawa H, Kataba H, Hirano T, Kato H, Matsuoka T and Mukai K: Coexpression of actin-related protein 2 and Wiskott-Aldrich syndrome family verproline-homologous protein 2 in adenocarcinoma of the lung. Clin Cancer Res *12*: 2449-2454, 2006.

33 Iwaya K, Oikawa K, Semba S, Tsuchiya B, Mukai Y, Otsubo T, Nagao T, Izumi M, Kuroda M, Domoto H and Mukai K: Correlation between liver metastasis of the colocalization of actin-related protein 2 and 3 complex and WAVE2 in colorectal carcinoma. Cancer Sci *98*: 992-999, 2007.

34 Iwaya K, Norio K and Mukai K: Coexpression of Arp2 and WAVE2 predicts poor outcome in invasive breast carcinoma. Mod Pathol *20*: 339-343, 2007.

35 Kaneda A, Kaminishi M, Sugimura T and Ushijima T: Decreased expression of the seven ARP2/3 complex genes in human gastric cancers. Cancer Lett *212*: 203-210, 2004.

Received November 2, 2012
Revised November 22, 2012
Accepted November 23, 2012