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Research article

DU-145 PROSTATE CARCINOMA CELLS THAT SELECTIVELY TRANSMIGRATE NARROW OBSTACLES EXPRESS ELEVATED LEVELS OF Cx43

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Abstract: The formation of aqueous intercellular channels mediating gap junctional intercellular coupling (GJIC) is a canonical function of connexins (Cx). In contrast, mechanisms of GJIC-independent involvement of connexins in cancer formation and metastasis remain a matter of debate. Because of the role of Cx43 in the determination of carcinoma cell invasive potential, we addressed the problem of the possible Cx43 involvement in early prostate cancer invasion. For this purpose, we analysed Cx43-positive DU-145 cell subsets established from the progenies of the cells most readily transmigrating microporous membranes. These progenies displayed motile activity similar to the control DU-145 cells but were characterized by elevated Cx43 expression levels and GJIC intensity. Thus, apparent links exist between Cx43 expression and transmigration potential of DU-145 cells. Moreover, Cx43 expression profiles in the analysed DU-145 subsets were not affected by intercellular contacts and chemical inhibition of GJIC during the transmigration. Our observations indicate that neither cell motility nor GJIC determines the transmigration efficiency of DU-145 cells. However, we postulate that selective transmigration of prostate cancer cells expressing elevated levels of Cx43 expression may be crucial for the "leading front" formation during cancer invasion.

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Abbreviations used: AGA – 18-α-glycyrrhetinic acid; ARCD – average rate of cell displacement; ASCM – average speed of cell movement; C_r – coupling ratio; Cx43 – connexin43; DMEM – Dulbecco modified Eagle's medium; FBS – fetal bovine serum; GJIC – gap junctional intercellular coupling

Key words: Cancer invasion, Cell heterogeneity, Cell motility, Cx43, Gap junctions, Metastasis, Prostate cancer, Transmigration

INTRODUCTION

Connexins (Cx) represent a family of membrane proteins that spontaneously form hexamers, called connexons. The primary function of connexons is the formation of aqueous channels between the adjacent cells, which mediate direct intercellular transfer of small (< 1.5 kDa) metabolites (gap junctional intercellular coupling; GJIC [1]). However, connexins and connexons affect a range of cellular processes in a manner independent of GJIC. For instance, Cx43 and unpaired Cx43 connexons were demonstrated to inhibit cell proliferation [2-5] and to enhance directed cell motility [6, 7] in a manner potentially dependent on their ability to interact with cytoskeletal components [8-10].

GJIC plays a crucial role in tissue homeostasis and its impairment in connexin-deficient cell populations is involved in the formation of neoplastic loci and primary tumours [11, 12]. On the other hand, connexins can enhance the invasive potential of cancer cells in a GJIC-dependent as well as GJIC-independent manner [13-15]. While GJIC between cancer and endothelial cells was shown to facilitate cancer cell penetration of the endothelial layer [16, 17], a GJIC-independent function of Cx43 in brain tumour invasion was reported [14]. These observations suggest the stage-specific function of connexins in the process of cancer development [15]. Impaired connexin expression and GJIC may facilitate early cancer development. However, the re-expression of connexins occurring within specific subsets of heterogeneous cancer cell populations results in the aberrant exchange of intercellular signals between normal and tumour cells, facilitating cancer invasion. In the light of recent data on coupling-independent functions of connexins [4, 18], the question arises about the role of connexins in the formation of cancer cell subpopulations constituting a “leading-front” of early invasion.

Heterogeneous cell populations [19, 20] constituting primary solid tumours comprise subsets of motile cells characterized by the capability of invading surrounding tissues and of metabolic coupling with normal cells. Their early selection seems crucial for subsequent formation of metastases. When recapitulated in vitro, such a selection enables identification of basic cellular properties crucial for early cancer progression [21]. In the present study, we describe an experimental approach enabling the assessment of heterogeneity of prostate carcinoma cells with regard to Cx43 expression and their invasive potential. We demonstrate that the progeny of heterogeneous DU-145 cells most readily transmigrating narrow obstacles is enriched in cells expressing elevated levels of Cx43 and increased GJIC competence. These data indicate that the selection of metastatic cancer cells capable of metabolic coupling with normal tissue cells may occur already during early cancer invasion.

MATERIAL AND METHODS

DU-145 cell culture and transmigration experiments

Human prostate carcinoma DU-145 cells were cultivated in DMEM-F12 HAM medium supplemented with 10% FBS (all from Sigma). When indicated, medium containing of 18-α-glycyrrhetinic acid (AGA; Sigma) – a blocker of GJIC – was added to the medium upon cell seeding. In transmigration experiments, DU-145 cells were seeded into chambers containing microporous membranes (Corning; pore diameter 8 μm; membrane diameter 6.5 mm) at the density of 300 cells/mm^2 in the medium alone or the medium supplemented with AGA and allowed to transmigrate for the next 48 hrs, i.e. the time sufficient for the first cells to precipitate onto the well bottom. Their progeny (DU-145_48) was propagated and used for endpoint experiments, along with the progeny of the cells that transmigrated between the 48^{th} and 72^{nd} hour of the experiment and precipitated onto the bottom of another well (DU-145_72 cells; see Fig. 2).

siRNA inhibition of Cx43 expression

DU-145 cells were seeded at a density of $7x10^4$ cells per well in a 12-well plate in antibiotic-free DMEM-F12 HAM medium supplemented with 10% FBS and grown overnight at 37°C before transfection. The cells were transfected with MISSION®esiRNA (114 pmol, Sigma) using Lipofectamine™2000 (Invitrogen) according to the manufacturer's protocol for 24 hrs. Afterwards, the medium was replaced by standard DMEM-F12 HAM supplemented with 10% FBS and gentamicin, and the efficiency of the inhibition of Cx43 expression was subsequently analysed using immunoblotting along with the estimation of the motility of transfected cells with time-lapse microscopy.

Time-lapse monitoring of movement of individual cells

The movement of DU-145 cells was time-lapse recorded for 4 hrs and the tracks of individual cells were determined from the series of changes in the cell centroid positions, pooled and analysed as previously described [22, 23]. The following parameters were estimated: (i) the total length of cell displacement (μm), i.e. the distance from the starting point directly to the cell's final position, (ii) the rate of cell displacement (μm/h), i.e. the distance from the starting point directly to the cell's final position/time of recording (4 hours), (iii) the speed of cell movement (μm/h), i.e. total length of cell trajectory/time of recording (4 hrs). Cell trajectories from no less than three independent experiments (number of cells > 50) were taken for the estimation of statistical significance by the non-parametric Mann-Whitney test $^{\#}p < 0.01$.

Immunocytochemistry, immunoblotting and GJIC analyses

Actin cytoskeleton architecture was analysed in formaldehyde-fixed, Triton X-100-solubilised DU-145 cells stained with TRITC-conjugated phalloidin (Sigma). For immunocytochemical analyses of intracellular localization of Cx43, cells were fixed with methanol:acetone (7:3, -20°C), labelled with rabbit

anti-Cx43 IgG (Sigma) and counterstained with Alexa 488-conjugated goat anti-rabbit IgG (Invitrogen, Carlsbad, CA) and 0.5 μg/ml bis-benzimide (Hoechst) [24]. Visualization of Cx43-positive plaques and semi-quantitative evaluation of their abundance at intercellular contacts was performed with a Leica DMIRE2 microscope. For Western blot analyses, DU-145 cell cultures were dissolved in lysis buffer and cellular proteins (30 μg/lane) were applied to 15% SDS-polyacrylamide gels, followed by transfer to nitrocellulose. Blots were exposed to primary rabbit polyclonal anti-Cx43 and mouse monoclonal anti-α-tubulin antibody (both from Sigma) followed by detection of the antibodies using HRP-labelled secondary antibodies (Invitrogen) and SuperSignal West Pico Substrate (Pierce, Rockford, IL) [25]. GJIC intensity was measured as previously described [26] with some modifications. Briefly, donor cells labelled with calcein (Molecular Probes) were plated onto monolayers of acceptor cells grown in Petri dishes at a ratio of l:50. The dynamics of calcein transfer from donor to acceptor cells was visualised using a Leica DM IRE2 time-lapse system and analysed with ImageJ freeware. For this purpose, values of the averaged coupling ratio (c_r), i.e. the number of calcein-positive recipient cells per donor cell, were elucidated 24, 30, 36 and 42 min. after donor cell seeding. At least 50 donor cells per Petri dish analysed in three independent experiments performed for each cell population were taken for the estimation of statistical significance by Student's t-test; * = $p < 0.05$.

RESULTS

Correlation between Cx43 plasmalemmal localization and transmigration activity of DU-145 cells

Immunofluorescence studies revealed heterogeneous expression levels and patterns of Cx43 localization in single DU-145 cells. Cx43 could be observed in perinuclear regions of their cytoplasm, in the cell protrusions and in plaques localized at intercellular contacts (Fig. 1A). Similarly, DU-145 cells displayed heterogeneous motile activity. This is shown in the circular diagram of cell trajectories (Fig. 1B) and the frequency histogram of cell displacements in sparse cultures (Fig. 1C), where the fractions of motile and immobile cells could be discriminated. Thus, the DU-145 cell line comprises sub-populations differing in cell motility and expression pattern of Cx43, two features involved in determination of the invasive potential of cancer cells.

Transmigration tests were further performed to establish the invasive subsets of DU-145 cells and to elucidate the correlation of Cx43 expression on their invasive potential. The DU-145_48 subset representing the progeny of the cells most readily transmigrating microporous membranes (2.24% of initial cell population; Fig. 2B) displayed elevated numbers of Cx43-positive plaques at cell-to-cell contacts (Fig. 2E cf. D for control DU-145 cell population).

Relatively high numbers of Cx43-positive plaques could also be observed in DU-145_72 cell populations (Fig. 2F cf. D, semi-quantitatively summarised in

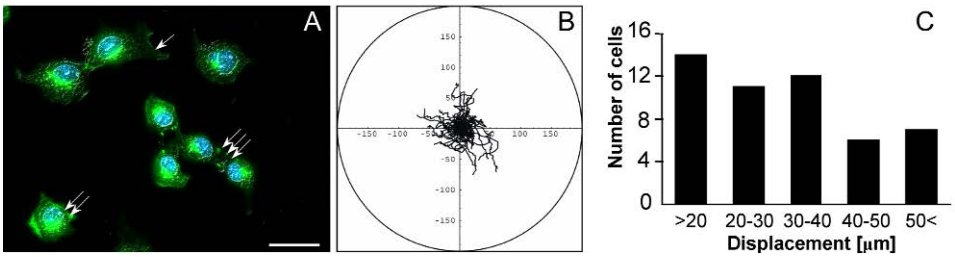

Fig. 1. Heterogeneity of Cx43 expression (A) and cell motility (B, C) in sparse DU-145 populations. Single, double and triple arrows indicate Cx43-negative and -positive cellular protrusions, and Cx43 plaques at intercellular contacts, respectively (A). Trajectories of DU-145 cells (registered for 4 hrs at 300 s time intervals) are presented in the form of a circular diagram (axis scale in μm) drawn with the initial point of each trajectory placed at the origin of the plot. The frequency histogram depicts the distribution of length values for displacement of DU-145 cells. Bar = 25 μm.

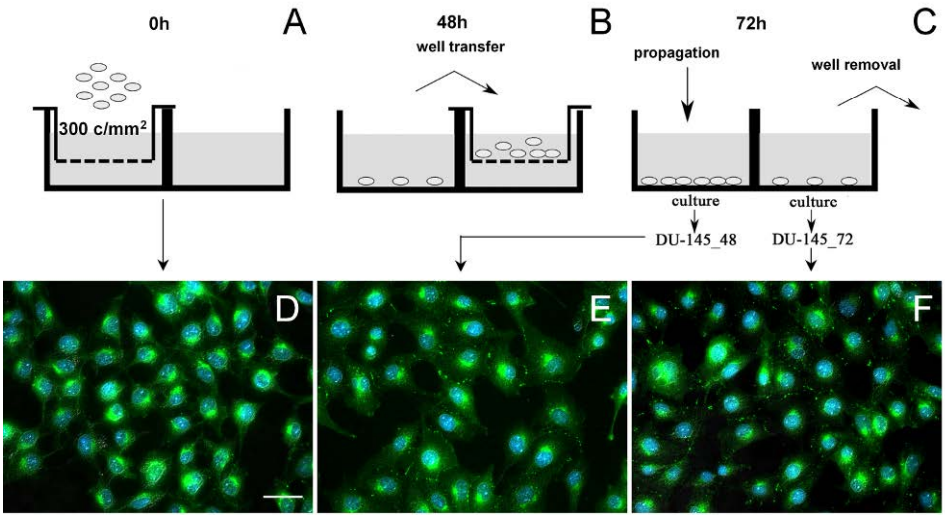

Fig. 2. Selective transmigration of DU-145 cells through microporous membranes results in the formation of subsets characterized by increased numbers of Cx43-positive plaques at intercellular contacts. DU-145 cells were seeded into the chambers at the density of 300 cells per mm^2 (A) and allowed to transmigrate for 48 hrs. Afterwards, the chambers were placed in another well (B) and cells were allowed to transmigrate for the next 24 hrs (C). The progenies of cells that managed to precipitate onto the bottoms of the wells were further analysed using fluorescence and time-lapse microscopy (see Fig. 4). DU-145_48 and DU-145_72 populations (E and F, respectively) were characterised by considerably higher abundance of Cx43-positive plaques than control cell populations (D, semi-quantitatively summarised in Tab. 1). Bar – 25 μm.

Tab. 1. Semi-quantitative analysis of the abundance of Cx43-positive plaques at intercellular contacts in human prostate carcinoma DU-145 cell sub-populations

Intensity of punctate Cx43-specific fluorescence		
Cell population		
DU-145	DU-145_48	DU-145_72
(+)	**(+++)**	**(++)**

(+) – < 2 plaques/cell; (++) – 2-6 plaques/cell; (+++) > 6 plaques /cell

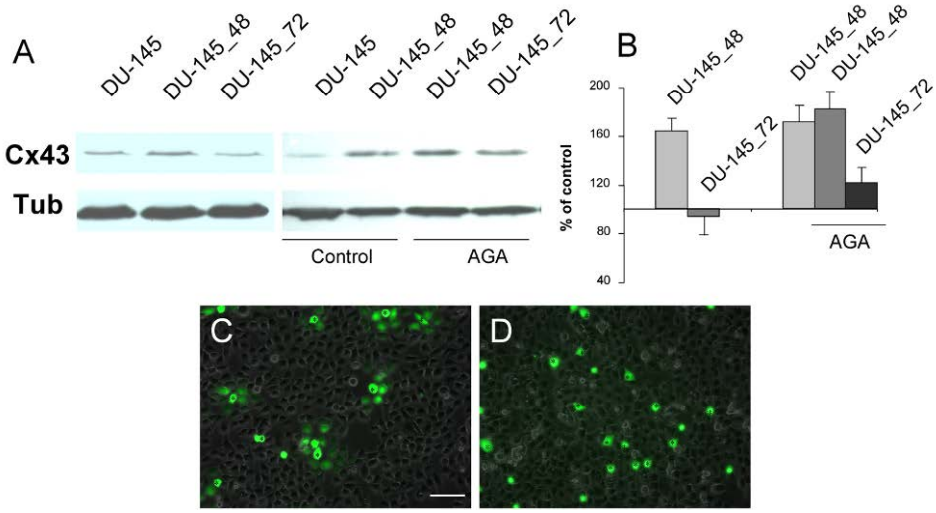

Fig. 3. Cx43 protein levels in the progenies of DU-145 cells transmigrating microporous membranes in control conditions and in the presence of GJIC blocker. Western blot analyses revealed increased Cx43 protein amounts in DU-145_48 but not DU-145_72 cell populations (A, left panel; quantitatively summarized in B). AGA-induced inhibition of GJIC, illustrated by the inhibition of calcein transfer (green) from donor (marked with asterisk) to acceptor cells (D, cf. C for control conditions; 30 min after donor cell seeding), exerted no effect on Cx43 expression in the progeny of transmigrating cells (A, right panel; quantitatively summarized in B). Bar – 100 μm.

Tab. 1) propagated from the progeny of the "second wave" of transmigrating DU-145 cells (2.42% of initial cell population; Fig. 2C). On the other hand, immunoblot analyses demonstrated increased Cx43 expression levels in DU-145_48 but not in DU-145_72 cell populations (Fig. 3A, left panel). These data indicate that the subpopulations of DU-145 cells characterized by elevated levels of plasmalemmal Cx43 preferentially transmigrate narrow obstacles.

Since transmigration experiments were performed in conditions partly securing physical isolation of the cells (300 cells/mm^2), it can be stated that the transmigration efficiency of DU-145 cells was not affected by the functional status of gap junctional channels. To substantiate this notion, transmigration tests were performed in the presence of 18-α-glycyrrhetinic acid (AGA). When

administered at the concentration of 100 μM, AGA completely inhibits GJIC between DU-145 cells throughout the incubation period (Fig. 3D, cf. C). As shown in Fig. 3A (right panel) and summarized in Fig. 3B, the progeny of DU-145 cells transmigrating in such conditions was enriched in cells expressing high levels of Cx43. Thus, preferential transmigration of DU-145 cells expressing elevated levels of Cx43 occurs in the presence of non-functional connexons.

Motility and GJIC in DU-145 cell subpopulations constituting a "leading front" of cancer invasion

To elucidate mutual interrelations between levels of Cx43 expression and the motility of DU-145 cells, analyses of the motility, morphology and actin cytoskeleton organisation of cells constituting the invasive subsets of DU-145 cells were performed. As shown by circular diagrams of cell trajectories (Fig. 4B, C) and dot plots depicting motile activity of single cells (Fig. 4E, F), invasive DU-145 cells were characterised by contact-stimulated and heterogeneous cell movement similar to that observed in control DU-145 cell populations (Fig. 4A, D, respectively, see Tab. 2). Furthermore, invasive and control cells displayed a similar diffuse F-actin organisation characterised by the accumulation of microfilaments

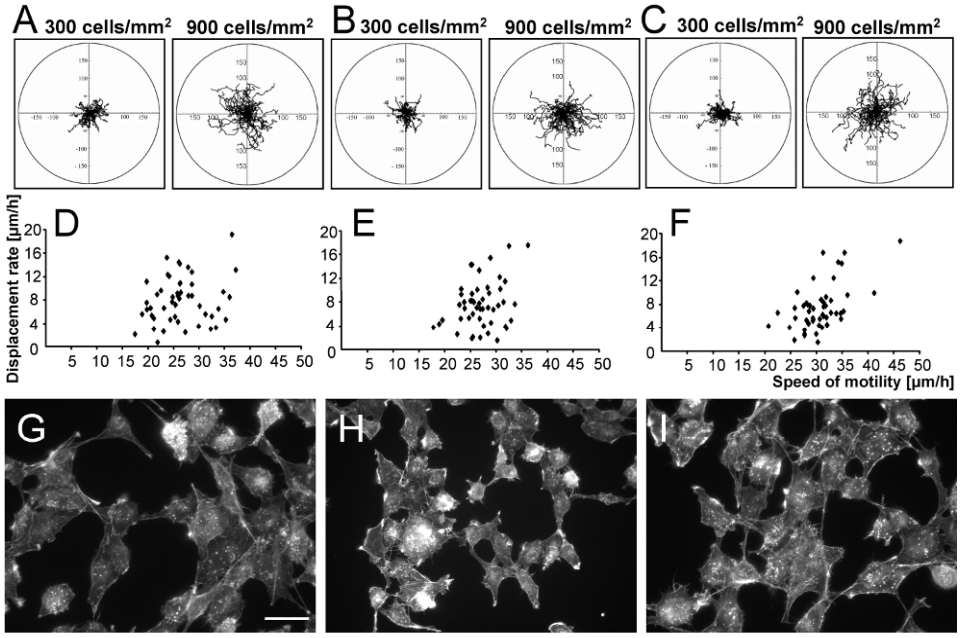

Fig. 4. Motile activity and actin cytoskeleton in DU-145 subsets differing in transmigration potential. DU-145_48 (B,E,H) and DU-145_72 (C, F, I) cells display motile activity (A-F), morphology and architecture of actin cytoskeleton (G-I) similar to control DU-145 cells (A, D, G) in both sparse and dense cultures. Cell trajectories (registered for 4 hrs at 300 s time intervals) are presented in the form of circular diagrams (axis scale in μm) drawn with the initial point of each trajectory placed at the origin of the plot. Dot plots show the distribution of displacement rates and speed of motility estimated for single cells. Bar – 25 μm.

Tab. 2. Summary of quantitative data on the motile activity of cells constituting the established DU-145 cell subsets.

Parameters (± SEM)	DU-145 (control)		DU-145_48		DU-145_72	
	300 cells/mm^2	900 cells/mm^2	300 cells/mm^2	900 cells/mm^2	300 cells/mm^2	900 cells/mm^2
Total length of cell displacement [μm][1)]	31.0 ± 2.2	61.91 ± 4.1	30.06 ± 2.3	67.57 ± 4.6	29.7 ± 2.2	60.9 ± 3.7
Average rate of cell displacement [μm/h][2)]	7.75 ± 0.6	15.48 ± 1.0	7.52 ± 0.6	16.89 ± 1.1	7.43 ± 0.5	15.23 ± 1.0
Average speed of cell movement [μm/h][3)]	26.43 ± 0.7	41.42 ± 0.9	27.08 ± 0.5	46.8 ± 1.1	30.69 ± 0.6*	43.66 ± 0.7

[1)] Total length of cell displacement is defined as the distance from the starting point direct to the cell's final position; [2)] Average rate of cell displacement is defined as total length of cell displacement from the starting point to the final cell position/time of recording (4 hrs); [3)] Average speed of cell movement is defined as total length of cell trajectory/time of recording (4 hrs). Statistically significant (Mann-Whitney test) probe vs. control (total DU-145); $^{\#}p < 0.01$.

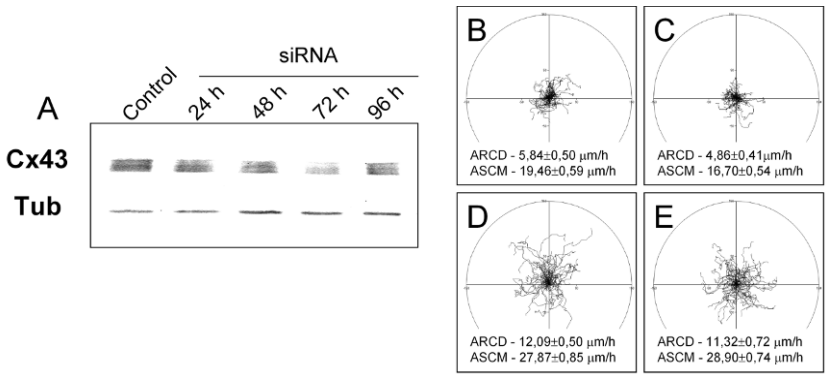

Fig. 5. Effect of the inhibition of Cx43 expression by siRNA (A) on DU-145 cell motility in sparse (B, C) and dense (D, E) cultures. siRNA transfected cells (C,E) displayed motility similar to control (B,D) cells. Cell trajectories (registered for 4 hours at 300 s time intervals) are presented in the form of circular diagrams (axis scale in μm) drawn with the initial point of each trajectory placed at the origin of the plot.

in the regions adjacent to cellular protrusions (Fig. 4G-I). These data demonstrate lack of a correlation between Cx43 expression and the motility of DU-145 cell subsets.

This notion was further substantiated by siRNA experiments which demonstrated that transient inhibition of Cx43 expression (Fig. 5A) exerted only a minute effect on control DU-145 cell motility. The motility of DU-145 cells analysed 72 hours after transfection, i.e. when the pronounced inhibition of Cx43 expression was observed, was similar to the motility of control cells regardless of their density (Fig. 5B-E). This observation indicates a lack of functional link between Cx43 expression and heterogeneous locomotion of DU-145 cells during the process of transmigration. Thus, neither motile activity of DU-145 cells nor differences in the architecture of their cytoskeleton can account for the correlation between the abundance of plasmalemmal fraction of Cx43 and DU-145 cell ability to penetrate narrow obstacles.

On the other hand, elevated numbers of Cx43-positive plaques at intercellular contacts between invasive DU-145 cells correlated with increased efficiency of GJIC in DU-145_48 and DU-145_72 cell populations (Fig. 6B and C, respectively; cf. A). This is illustrated by a more than three-fold increase of the average c_r in DU-145_48 cell populations compared to control DU-145 cells 42 min. after donor cell seeding (Fig. 6D). These data indicate that transmigration of DU-145 cells results in the formation of well-coupled DU-145 subsets.

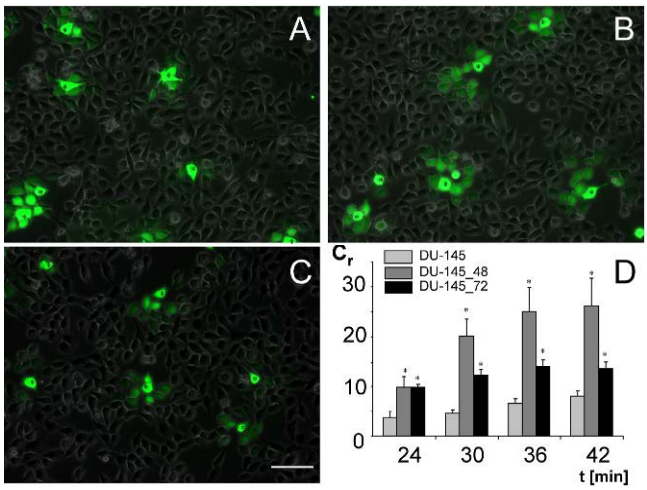

Fig. 6. Gap junctional coupling in the populations of control DU-145 cells (A), DU-145_48 (B) and DU-145_72 (C) cells. Enhanced GJIC within DU-145_48 (B) and DU-145_72 (C) compared to control cell populations (A) is illustrated by intensified transfer of calcein (green) from donor (marked with asterisk) to acceptor cells 20 min. after donor cell seeding and c_r values calculated for the analysed cell populations at different co-incubation times (D). *$p < 0.05$ as determined by paired Student's t-test. Scale bar = 100 μm.

DISCUSSION

During cancer progression, subpopulations of "invasive" cells are formed within an expanding and phenotypically heterogeneous tumour cell population [27]. Due to their specific properties, such as high mechanical elasticity, active motility and the capability of tissue remodelling and of communication with local microenvironments [28-30], these cells are apt to effectively cross tissue barriers [31, 32] and to form a "leading front" of cancer invasion. Heterogeneity of cancer cells observed *in vivo* was also found within *in vitro* cultured cancer cell populations [13, 32-34]. We demonstrate such heterogeneity of DU-145 cell populations with regard to Cx43 expression. More importantly, we show that the capability of DU-145 cells to transmigrate narrow obstacles coincides with high expression levels of Cx43 in these cells. Thus, selective cell transmigration in the conditions mimicking early cancer invasion results in the formation of cellular subsets readily forming functional gap junctions. Due to the crucial role

of metabolic coupling between cancer and normal cells in cancer progression [17, 35], it may increase the efficiency of cancer cell dissemination from primary tumours.

Transmigration tests were performed in conditions securing the physical isolation of the cells. Moreover, pharmacological block of gap junctional coupling exerted no effect on the Cx43 expression profile in the progeny of transmigrating cells. Thus, a fraction of DU-145 cells can preferentially transmigrate narrow obstacles in the presence of non-functional connexons. The question arises about the possible ways of Cx43 participation in the determination of DU-145 cell transmigration potential. A GJIC-independent function of Cx43 in the regulation of cell locomotion has been previously described [6, 14]. However, we did not observe any correlation between the expression and function of Cx43 and motility of DU-145 cells. The progeny of transmigrating cells, while expressing elevated levels of Cx43, displayed motility similar to control cells. This observation indicates that if Cx43 is directly involved in the determination of DU-145 cell transmigration potential, it exerts this effect in a manner independent of cell motility. In parallel, it remains in opposition to previous reports describing effects of Cx43 on cell adhesion and directed motility [3, 4, 6, 10, 14, 18].

However, the processes of cell migration on planar surfaces and cell transmigration through narrow obstacles may be governed by discrete regulatory systems [30, 36]. It is possible that subtle changes in cytoskeletal function that facilitate cellular susceptibility to mechanical distortions [29, 37] and/or directed cell motility through narrow obstacles may accompany elevated Cx43 expression. This explanation is consistent with the reports on the interactions of connexins with cytoskeleton proteins [4, 10], resulting in a protective effect against mechanical injury of cancer cells [8]. Importantly, it would explain the formation of cancer cell subpopulations characterised by heterogeneous motile activity on planar substrata and displaying high competence for GJIC – a parameter crucial for penetration of tissue barriers such as endothelium [15-17, 35, 38].

While direct involvement of Cx43 in the transmigration of DU-145 cells remains a matter of speculation, the coincidence between plasmalemmal localisation of Cx43 and transmigration potential of DU-145 cells may have strong implications for our understanding of its involvement in cancer progression. Cancer cells have developed a range of strategies facilitating their penetration of the connective tissue and the process of intra- and extravasation. They remain under the control of versatile regulatory systems including cell adhesion and shape, function of the cytoskeleton [30] and rearrangements of the cell “niche” [39, 40] resulting in the formation of migratory trails [30, 41]. Our observations support the notions about Cx43 as an important element of this “puzzle”. They indicate that the selective migration of cancer cell subpopulations competent for GJIC with normal cells and thus more apt to invade healthy tissues may occur already at early stages of cancer invasion.

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REFERENCES

1. Sohl, G. and Willecke, K. Gap junctions and the connexin protein family. **Cardiovasc. Res.** 62 (2004) 228-232.
2. Zhang, Y.W., Kaneda, M. and Morita, I. The gap junction-independent tumor-suppressing effect of connexin 43. **J. Biol. Chem.** 278 (2003) 44852-44856.
3. Omori, Y., Li, Q., Nishikawa, Y., Yoshioka, T., Yoshida, M., Nishimura, T. and Enomoto, K. Pathological significance of intracytoplasmic connexin proteins: implication in tumor progression. **J. Membr. Biol.** 218 (2007) 73-77.
4. Cronier, L., Crespin, S., Strale, P.O., Defamie, N. and Mesnil, M. Gap junctions and cancer: new functions for an old story. **Antioxid. Redox. Signal.** 11 (2009) 323-338.
5. Ionta, M., Ferreira, R.A., Pfister, S.C. and Machado-Santelli, G.M. Exogenous Cx43 expression decrease cell proliferation rate in rat hepatocarcinoma cells independently of functional gap junction. **Cancer Cell Int.** 9 (2009) 22.
6. Elias, L.A., Wang, D.D. and Kriegstein, A.R. Gap junction adhesion is necessary for radial migration in the neocortex. **Nature** 448 (2007) 901-907.
7. Wiencken-Barger, A.E., Djukic, B., Casper, K.B. and McCarthy, K.D. A role for Connexin43 during neurodevelopment. **Glia** 55 (2007) 675-686.
8. Lin, J.H., Yang, J., Liu, S., Takano, T., Wang, X., Gao, Q., Willecke, K. and Nedergaard, M. Connexin mediates gap junction-independent resistance to cellular injury. **J. Neurosci.** 23 (2003) 430-441.
9. Xu, X., Francis, R., Wei, C.J., Linask, K.L. and Lo, C.W. Connexin 43-mediated modulation of polarized cell movement and the directional migration of cardiac neural crest cells. **Development** 133 (2006) 3629-3639.
10. Olk, S., Zoidl, G. and Dermietzel, R. Connexins, cell motility, and the cytoskeleton. **Cell Motil. Cytoskeleton** 66 (2009) 1000-1016.
11. Laird, D.W. Life cycle of connexins in health and disease. **Biochem. J.** 394 (2006) 527-543.
12. Trosko, J.E. Gap junctional intercellular communication as a biological "Rosetta stone" in understanding, in a systems biological manner, stem cell

behavior, mechanisms of epigenetic toxicology, chemoprevention and chemotherapy. **J. Membr. Biol.** 218 (2007) 93-100.
13. Miekus, K., Czernik, M., Sroka, J., Czyz, J. and Madeja, Z. Contact stimulation of prostate cancer cell migration: the role of gap junctional coupling and migration stimulated by heterotypic cell-to-cell contacts in determination of the metastatic phenotype of Dunning rat prostate cancer cells. **Biol. Cell** 97 (2005) 893-903.
14. Bates, D.C., Sin, W.C., Aftab, Q. and Naus, C.C. Connexin43 enhances glioma invasion by a mechanism involving the carboxy terminus. **Glia** 55 (2007) 1554-1564.
15. Czyz, J. The stage-specific function of gap junctions during tumourigenesis. **Cell Mol. Biol. Lett.** 13 (2008) 92-102.
16. Zhang, W., DeMattia, J.A., Song, H. and Couldwell, W.T. Communication between malignant glioma cells and vascular endothelial cells through gap junctions. **J. Neurosurg.** 98 (2003) 846-853.
17. Pollmann, M.A., Shao, Q., Laird, D.W. and Sandig, M. Connexin 43 mediated gap junctional communication enhances breast tumor cell diapedesis in culture. **Breast Cancer Res.** 7 (2005) R522-R534.
18. Prochnow, N. and Dermietzel, R. Connexons and cell adhesion: a romantic phase. **Histochem. Cell Biol.** 130 (2008) 71-77.
19. Boiko, A.D., Razorenova, O.V., van de, R.M., Swetter, S.M., Johnson, D.L., Ly, D.P., Butler, P.D., Yang, G.P., Joshua, B., Kaplan, M.J., Longaker, M.T. and Weissman, I.L. Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. **Nature** 466 (2010) 133-137.
20. Visvader, J.E. Cells of origin in cancer. **Nature** 469 (2011) 314-322.
21. Wysoczynski, M., Miekus, K., Jankowski, K., Wanzeck, J., Bertolone, S., Janowska-Wieczorek, A., Ratajczak, J. and Ratajczak, M. Z. Leukemia inhibitory factor: a newly identified metastatic factor in rhabdomyosarcomas. **Cancer Res.** 67 (2007) 2131-2140.
22. Sroka, J., Kaminski, R., Michalik, M., Madeja, Z., Przestalski, S. and Korohoda, W. The effect of triethyllead on the motile activity of walker 256 carcinosarcoma cells. **Cell Mol. Biol. Lett.** 9 (2004) 15-30.
23. Sroka, J., Antosik, A., Czyz, J., Nalvarte, I., Olsson, J.M., Spyrou, G. and Madeja, Z. Overexpression of thioredoxin reductase 1 inhibits migration of HEK-293 cells. **Biol. Cell** 99 (2007) 677-687.
24. Czyz, J., Guan, K., Zeng, Q., and Wobus, A.M. Loss of beta1 integrin function results in upregulation of connexin expression in embryonic stem cell-derived cardiomyocytes. **Int. J. Dev. Biol.** 49 (2005) 33-41.
25. Daniel-Wojcik, A., Misztal, K., Bechyne, I., Sroka, J., Miekus, K., Madeja, Z. and Czyz, J. Cell motility affects the intensity of gap junctional coupling in prostate carcinoma and melanoma cell populations. **Int. J. Oncol.** 33 (2008) 309-315.
26. Czyz, J., Irmer, U., Schulz, G., Mindermann, A. and Hulser, D.F. Gap-junctional coupling measured by flow cytometry. **Exp. Cell Res.** 255 (2000) 40-46.

27. Sottoriva, A., Verhoeff, J.J., Borovski, T., McWeeney, S.K., Naumov, L., Medema, J.P., Sloot, P.M. and Vermeulen, L. Cancer stem cell tumor model reveals invasive morphology and increased phenotypical heterogeneity. **Cancer Res.** 70 (2010) 46-56.
28. Baran, B., Bechyne, I., Siedlar, M., Szpak, K., Mytar, B., Sroka, J., Laczna, E., Madeja, Z., Zembala, M. and Czyz, J. Blood monocytes stimulate migration of human pancreatic carcinoma cells in vitro: the role of tumour necrosis factor - alpha. **Eur. J. Cell Biol.** 88 (2009) 743-752.
29. Kumar, S. and Weaver, V.M. Mechanics, malignancy, and metastasis: the force journey of a tumor cell. **Cancer Metastasis Rev.** 28 (2009) 113-127.
30. Friedl, P. and Wolf, K. Plasticity of cell migration: a multiscale tuning model. **J. Exp. Med.** 207 (2010) 11-19.
31. Gupta, G.P. and Massague, J. Cancer metastasis: building a framework. **Cell** 127 (2006) 679-695.
32. Langley, R.R. and Fidler, I.J. Tumor cell-organ microenvironment interactions in the pathogenesis of cancer metastasis. **Endocr. Rev.** 28 (2007) 297-321.
33. Watanabe, N., Dickinson, D.A., Krzywanski, D.M., Iles, K.E., Zhang, H., Venglarik, C.J., and Forman, H.J. A549 subclones demonstrate heterogeneity in toxicological sensitivity and antioxidant profile. **Am. J. Physiol Lung Cell Mol. Physiol** 283 (2002) L726-L736.
34. Blick, T., Widodo, E., Hugo, H., Waltham, M., Lenburg, M.E., Neve, R.M. and Thompson, E.W. Epithelial mesenchymal transition traits in human breast cancer cell lines. **Clin. Exp. Metastasis** 25 (2008) 629-642.
35. Ito, A., Katoh, F., Kataoka, T.R., Okada, M., Tsubota, N., Asada, H., Yoshikawa, K., Maeda, S., Kitamura, Y., Yamasaki, H. and Nojima, H. A role for heterologous gap junctions between melanoma and endothelial cells in metastasis. **J. Clin. Invest.** 105 (2000) 1189-1197.
36. Huang, S. and Ingber, D.E. Cell tension, matrix mechanics, and cancer development. **Cancer Cell** 8 (2005) 175-176.
37. Suresh, S. Biomechanics and biophysics of cancer cells. **Acta Biomater.** 3 (2007) 413-438.
38. Kanczuga-Koda, L., Sulkowski, S., Lenczewski, A., Koda, M., Wincewicz, A., Baltaziak, M. and Sulkowska, M. Increased expression of connexins 26 and 43 in lymph node metastases of breast cancer. **J. Clin. Pathol.** 59 (2006) 429-433.
39. Iwasaki, H. and Suda, T. Cancer stem cells and their niche. **Cancer Sci.** 100 (2009) 1166-1172.
40. Voog, J. and Jones, D.L. Stem cells and the niche: a dynamic duo. **Cell Stem Cell** 6 (2010) 103-115.
41. Friedl, P., Hegerfeldt, Y. and Tusch, M. Collective cell migration in morphogenesis and cancer. **Int. J. Dev. Biol.** 48 (2004) 441-449.