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Overexpression of dnIKK in mesenchymal stem cells leads to increased migration and decreased invasion upon TNFα stimulation

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ABSTRACT

IκB kinase 2 (IKK-2) mediates tumor necrosis-factor α (TNFα) induced invasion of human mesenchymal stem cell (hMSC) to sites of tissue injury. Suppressing IKK-2 activity leads to reduced expression of proteolytic enzymes and impaired invasive capacity. In order to further reveal mechanisms of hMSC recruitment, we here aimed to analyse the impact of IKK-2 on two-dimensional migration upon TNFα stimulation in contrast to three-dimensional invasion. An immortalized hMSC line (SCP-1) was transduced with a dominant-negative mutant of IκB kinase 2 (SCP-1 dnIKK). Migration was assessed using a linear-gradient chemotaxis chambers by time-lapse analysis. Invasive capacity through human extracellular matrix was analysed using transwell invasion assays. RT-PCR confirmed increased IKK-2 expression levels in SCP-1 dnIKK cells, while TNFα receptor I and II expression was not altered. Invasion upon TNFα stimulation was significantly reduced by 78% in SCP-1 dnIKK. In contrast, migration was significantly increased, represented by a 60% elevated forward migration index and a 2.1-fold higher mean dislocation of the center of mass towards TNFα. In conclusion, our data confirms the impact of IKK-2 in TNFα dependent hMSC recruitment. Interestingly, reducing IKK-2 function increases two-dimensional migration towards TNFα, while invasive capacity is impaired. These findings contribute to a deeper understanding of MSC's biological properties orchestrating the complex processes of stem cell recruitment and homing.

1. Introduction

Human mesenchymal stem cells (hMSC) represent the continuous cellular source for tissue repair and regeneration. The regenerative potential of hMSC is mainly based on their high self-renewal capacity paired with the ability to differentiate into various tissues including bone, cartilage and muscle [1]. Recruitment of hMSC to the sites of tissue injury requires active migration towards chemokines or cytokines and invasion by proteolytic interaction with the extracellular matrix [1–3]. *In vitro* studies on invasion are routinely carried out using three-dimensional (3-D) assays with extracellular matrix (ECM) barriers. Experimental data have shown that tumor necrosis-factor α (TNFα) is a strong stimulus for hMSC invasion [4,5]. As a ligand of TNF receptor I (p55) and II (p75), TNFα activates the canonical nuclear factor kappa B (NFκB) pathway. In unstimulated cells, the binding of NFκB to IκB in the cytoplasm prevents its translocation into the nucleus [6]. Upon stimulation, specific IκB kinases (IKK-1 and -2) phosphorylate IκB, causing its rapid degradation by proteasomes [7,8]. The release of NFκB from IκB results in the transfer of NFκB into the nucleus, where it binds to the promoter regions of its target genes. IKK-2 is described as a rate-limiting step of the NFκB pathway [9] and is crucial for TNFα-mediated invasion of hMSC [4]. Invasion through extracellular matrix requires upregulation of ECM binding receptors and proteolytic enzymes. Consequently, CD44 and matrix metalloproteinase (MMP) 9 upregulation is found in hMSC upon TNFα mediated invasion [4,5]. Reduction of IKK-2 activity inhibits upregulation of these genes, resulting in impaired invasive capacity.

Beside the observed upregulation of proteolytic enzymes and the increase of invasion capacity, TNFα is also known to be a strong chemoattractant in two-dimensional (2-D) migration [10,11]. Cell migration is broadly scrutinized in *in vitro* studies as directed cell movement on different surfaces [12]. It results from a three-step cycle of polarized cell extension and substrate binding through leading pseudopods, followed by actin-based contraction of the cell body and release of adhesive bonds at the trailing edge [13]. However, it is unknown whether TNFα induced 2-D migration of

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hMSC is also mediated via IKK-2. Therefore, the aim of the present study was to evaluate the involvement of IKK-2 on 2-D migration of hMSC upon TNFα stimulation. Elucidating the impact of IKK-2 on this distinct cellular process may contribute to a more profound understanding of cytokine guided hMSC recruitment to the sites of tissue injury.

2. Materials and methods

2.1. Cells and cell culture

Experiments were carried out using a single-cell-derived human mesenchymal stem cell line expressing hTERT after lentiviral gene transfer, termed SCP-1. Previous studies proved this cell line to be a reliable, standardized and well-characterized cell model analyzing mechanisms of hMSC signal transduction and biological response [14]. SCP-1 cells were additionally transduced with the dominant-negative mutant (K44A) IKK-2 (SCP-1 dnIKK) according to our previously published protocol [2]. Cells were cultured in MEM Alpha GlutaMAX culture media (Invitrogen, Germany) supplemented with 10% FBS (Sigma–Aldrich, Germany) and 1% penicillin/streptomycin in a humidified incubator at 5% CO_2 and 37 °C. SCP-1 cells were utilized between the 74–85 passage. Medium was replaced twice a week while a cell density of 60% was never exceeded to prevent spontaneous differentiation.

2.2. PCR

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany) and according to the manufacturer's protocol. RNA of untransduced hMSC served as a control. CDNA synthesis was carried out using the First-Strand cDNA Synthesis Kit (Invitrogen, Germany). RT-PCR was performed utilizing Taq DNA polymerase (Qiagen) and normalized to GAPDH expression levels. Primer pairs, cycle numbers and annealing temperatures for selected genes analysed in this study have been published previously [4,14,15]. PCR was performed in a PTC-200 thermal cycler (MJ Research, Germany). PCR products were subjected to 1.8% agarose gel electrophoresis and visualized by a digital scanning system (Intas Science Imaging Instruments, Germany).

2.3. Immunocytochemistry

SCP-1 and SCP-1 dnIKK cells were seeded in a concentration of 2×10^4 cells/cm^2 on sterile glass slides and incubated for 72 h. For the last 24 h cells were stimulated with TNFα (50 ng/ml; Biomol, Germany). After fixation with 4% paraformaldehyde, cells were blocked with 2% bovine serum albumin for 1 h. Primary antibody against NFκB p65 (Santa Cruz, USA) and secondary antibody conjugated to FITC (Dianova, Germany) were used in concentrations of 0.8 μg/ml and 2.5 μg/ml, respectively. Antibody incubations were carried out for 30 min at room temperature. Negative controls were performed on the same slide by omitting the primary antibody. Photomicrographs were taken by an axiocam MR mounted on an Axioskope2 microscope (Zeiss, Germany).

2.4. Invasion assay

The invasive capacity of SCP-1 cells was analysed using a transwell invasion assay. Cell culture inserts (HTS FluoroBlok) with a light-tight polyethylene terephthalate membrane and 8.0 μm pore size were placed in a 24-well Cell Culture Insert Companion plate (both BD Bioscience, USA). Prior to seeding SCP-1 cells on the apical side of the membrane, filters were coated with 10 μg of human extracellular matrix (BD Biosciences, USA). The lower compartment of the invasion chamber was filled with 600 μl serum free culture media containing TNFα in a concentration of 50 ng/ml. Coated filter inserts were placed into the wells forming the upper compartment. SCP-1 cells (5×10^3) were susn1x1pended in 200 μl serum free culture media and seeded into the upper compartment of the invasion chamber. The invasion chambers were incubated for 12 h under standard culture conditions. After incubation, cells and ECM on the top surfaces of the filters were wiped off. Cells that had migrated into the lower compartment and attached to the lower surface of the filter were counted in 25 randomly chosen fields of view (909 × 909 μm) after staining with 10 mM carboxyfluorescein diacetate succinimidyl ester CFDA-SE (Invitrogen, Germany). Three independent invasion experiments were performed in triplicates.

2.5. Migration assay

The assessment of 2-D directed migration in a linear gradient was carried out in collagen IV coated μ-slide chemotaxis chambers purchased from IBIDI, Germany [16,17]. Cultivation channels were seeded with cells in a concentration of 1×10^6/ml according to the manufacturer's protocol and incubated for 3 h. Subsequently, the culture medium was washed out and replaced by serum free medium. Microslide reservoirs were filled with serum free culture medium and TNFα gradient was applied with a peak concentration of 50 ng/ml. Serum free culture medium served as a control group. Microscopic imaging was initiated after 1 h stabilization and equilibration of the chemotaxis gradient. Time lapse imaging was carried out over 15 h in a controlled bio-chamber (Pecon, Germany) at 5% CO_2 and 37 °C. Images were automatically acquired every 20 min by AxioVision Software (Zeiss, Germany). Microscopy was performed using an inverted Axiovert S100 equipped with a Plan-Neofluar 10×/0.3 Ph1 objective (Zeiss, Germany). Thirty randomly chosen cells were analysed in three independent experiments. Cell tracking was performed using ImageJ software (open source by NIH, USA) including the plugins "Manual Tracking" (Fabrice Cordelières, France) and "Chemotaxis and Migration Tool" (IBIDI Integrated BioDiagnostics, Germany). Cell coordinates were retrieved by marking the nuclei in every captured frame. The obtained data was further processed, visualized and plotted, thereby allowing the calculation of covered distances, directionality and velocity of each cell between two frames. The migration towards the chemokine gradient was then quantified by means of forward migration index (FMI) and center of mass (COM). The FMI is the ratio of covered distance along the chemokine gradient and the cell's overall path length. The COM is the mean of all cells' endpoint coordinates along the chemokines gradient. Statistical calculations were performed using GraphPad Prism v5.02 (Graphpad Software Inc., USA). Significance was fixed at $p < 0.05$ and tested by two-tailed unpaired t-test or Bonferroni's Multiple Comparison Test.

3. Results

3.1. Lentiviral transduction and characterization of SCP-1 dnIKK cells

Phase contrast observation during cell culturing revealed no morphological differences between SCP-1 and SCP-1 dnIKK cells (Fig. 1A). Semi-quantitative RT-PCR was performed to demonstrate the efficiency of hTERT and dnIKK lentiviral transduction as well as to investigate potential changes in TNFα receptor expression. SCP-1 cells highly expressed hTERT and expression levels remained unchanged after transduction of dnIKK-2. No hTERT expression was detected in untransduced hMSC. A basal expression of IKK-2 was found in hMSC and SCP-1, whereas IKK-2 expression was

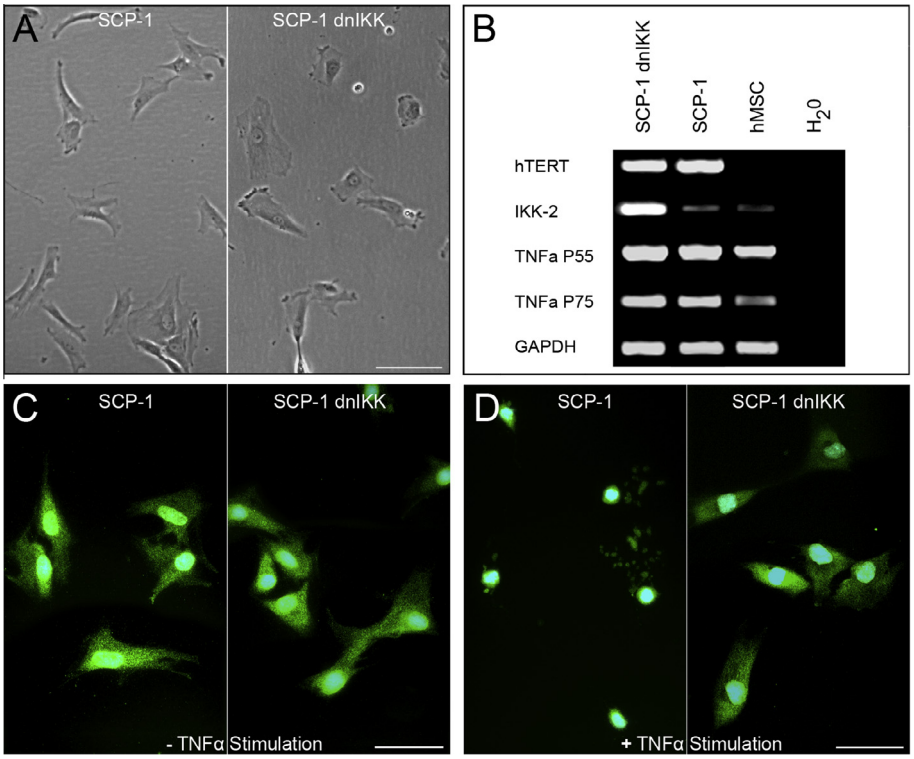

Fig. 1. Characterization of hMSC before and after lentiviral dnIKK-2 transduction. (A) Phase contrast microscopy revealed no alterations of the cell morphology in SCP-1 cell due to dnIKK overexpression. (B) RT-PCR demonstrated strong hTERT expression in SCP-1 and SCP-1 dnIKK cells, while it is not expressed in untransduced hMSC. IKK-2 expression was notably higher in SCP-1 dnIKK cells compared to the basal expression in hMSC and SCP-1 cells. TNFα receptors (P55 and P75) were equally expressed in all cells, independent of dnIKK-2 overexpression. (C) Immunohistochemical staining of NF-κB (p65) showed its primary location within the cytoplasm of unstimulated SCP-1 and SCP-1 dnIKK cells. (D) TNF-α stimulation resulted in a translocation of p65 into the nucleus of SCP-1 cells, while this process was blocked in SCP-1 dnIKK cells (Bars = 50 μm).

notably higher in SCP-1 dnIKK. TNFR-1 and TNFR-2 were equally expressed in SCP-1 and SCP-1 dnIKK cells. Expression levels in untransduced hMSC were marginally lower. Thus, dnIKK-2 transduction had no effect on TNFα receptor expression (Fig. 1B). PCR results were reproduced three independent times.

Immunocytochemical staining against the p65 subunit of the inducible transcription factor NFκB was carried out to demonstrate the functional effect on signal transduction due to dnIKK overexpression. Therefore, staining procedures were performed without and after TNFα stimulation. Unstimulated SCP-1 and SCP-1 dnIKK cells showed a homogenously strong expression of p65 located in the cytoplasm (Fig. 1C). Stimulation of SCP-1 cells with 50 ng/ml TNFα for 24 h resulted in a nuclear translocation of the p65 subunit. This translocation was completely blocked in SCP-1 dnIKK cells (Fig. 1D).

3.2. Effect of IKK-2 on invasion in TNFα stimulated SCP-1 cells

3-D transwell invasion assays across an extracellular matrix barrier were performed to quantify the invasion capacity of SCP-1 and SCP-1 dnIKK towards TNF-α (50 ng/ml). Without TNFα stimulation a mean number of 0.8 (SD ± 1.1) SCP-1 and 1.6 (SD ± 1.8) SCP-1 dnIKK cells invaded across the ECM barrier into the lower compartment. There were no significant differences in basal invasion rates. Upon TNFα stimulation the mean number of invaded SCP-1 cells increased to 230 (SD ± 171), while only a mean number of 50.7 (SD ± 27.1) SCP-1 dnIKK cells invaded across the ECM barrier. Blocking the NF-κB signal transduction by dnIKK-2 caused an approximately 80% decreased invasion towards TNF-α. This reduction of invasion capacity was significant with $p < 0.05$ in Bonferroni's Multiple Comparison Test (Fig. 3).

3.3. Effect of IKK-2 on migration in TNFα stimulated SCP-1 cells

Migration assays were carried out to evaluate the impact of IKK-2 on 2-D migration of SCP-1 and SCP-1 dnIKK cells upon TNFα stimulation. Initially, the responsiveness of SCP-1 cells was affirmed in a linear TNFα gradient and compared to a serum free control group (Fig. 3). SCP-1 cells migrated towards the TNFα gradient (peak concentration 50 ng/ml) with a center of mass (COM) displacement of 30 μm (SD ± 3 μm) and a forward migration index (FMI) of 0.13 (SD ± 0.02). Medium control revealed −5 μm (SD ± 9.6 μm) and −0.03 (SD ± 0.03 μm), respectively. Both differences were significant.

Afterwards, the migration capacity of SCP-1 dnIKK against a TNFα gradient was quantified and compared to SCP-1 cells (Fig. 4). Media control was subtracted in both groups. Accordingly, SCP-1 cells migrated with a mean COM displacement of 35 μm (SD ± 9.6 μm) towards the TNFα gradient. SCP-1 dnIKK cells revealed a significantly higher migration activity with a center of mass displacement of 74 μm (SD ± 14.5 μm). The FMI was 0.16 (SD ± 0.03) for SCP-1 cells and 0.27 (SD ± 0.05) for SCP-1 dnIKK cells. This increase of chemotactical response towards TNFα was significant ($p < 0.05$). Results were obtained in three independent experiments.

4. Discussion

Regeneration of mesenchymal tissues requires the recruitment of mesenchymal stem cells as the continuous cellular source for tissue repair. This process necessitates directed cell migration and invasion across extracellular matrix barriers [18,19]. TNFα, among other proinflammatory cytokines, is released at the sites of injury, attracting hMSC and promoting invasion by upregulation

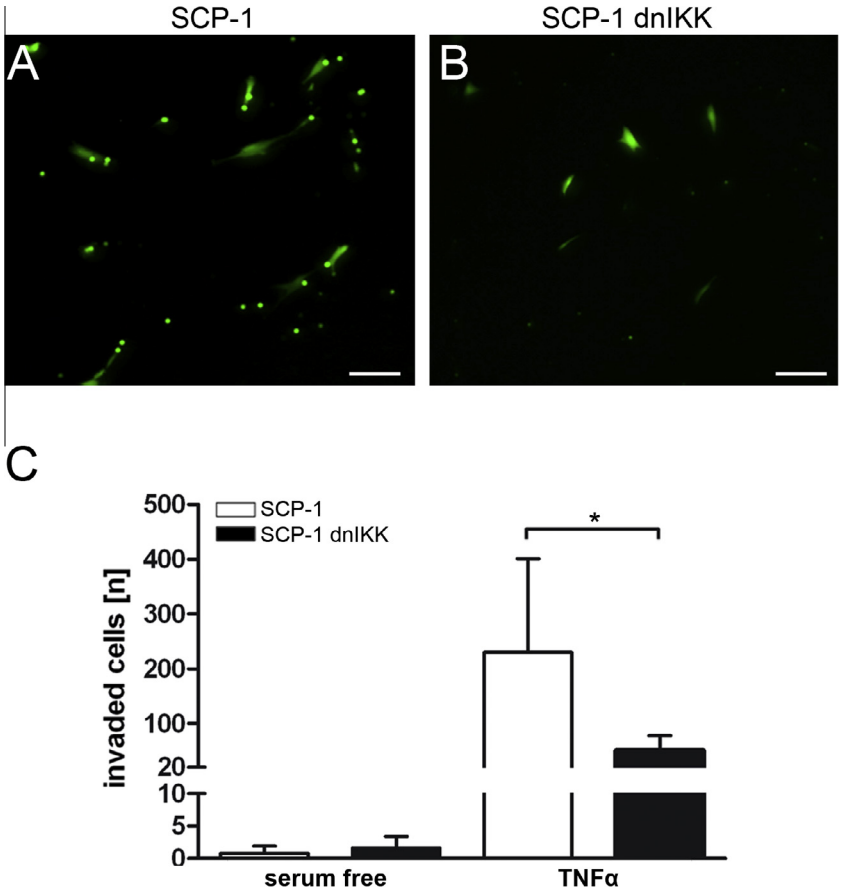

Fig. 2. Invasion across an extracellular matrix barrier upon TNFα stimulation. (A, B) Fluorescence staining of SCP-1 and SCP-1 dnIKK cells on the lower side of the porous insert after 12 h of incubation with 50 ng/ml TNFα. (C) Invasion towards the TNFα gradient is significantly reduced in SCP-1 dnIKK cells compared to SCP-1 cells. Serum free control groups showed very low levels of invasion activity. Data are presented as mean numbers of invaded cells ± SD of three independent experiments (Bars = 50 μm, $^{*}p < 0.05$).

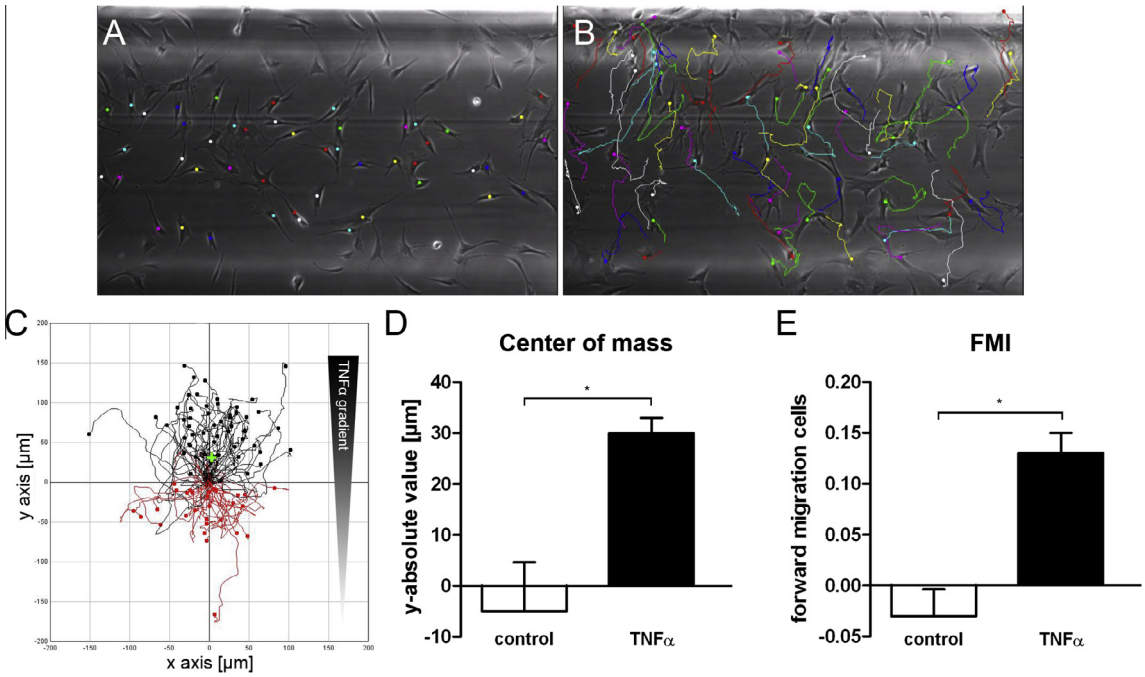

Fig. 3. Chemotactical response of SCP-1 cells towards a TNFα gradient on μ-slide chambers. Cell nuclei were marked at the start point of the time lapse observation and tracked every 20 min resulting in the accumulative cell paths after an observation period of 15 h (A, B) Exemplary observation with cell tracks visualized by colored lines at t = 0 and t = 15 h. (C) Chemotaxis scatter plot presents cell paths towards the TNFα gradient in black and the center of mass as a green cross. (D, E) Comparing the center of mass (COM) and the forward migration index (FMI) revealed the strong migratory response of SCP-1 cells towards TNFα. Data are presented as mean ± SD of three independent experiments (Bars = 100 μm, $^{*}p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

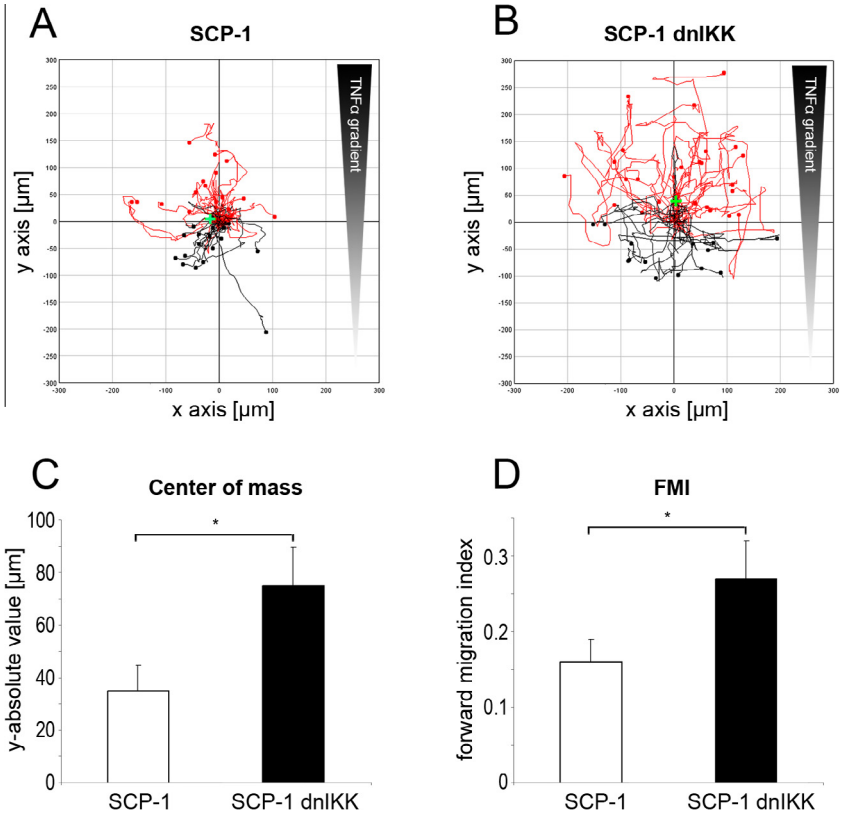

Fig. 4. Migration on µ-slide chamber upon TNFα stimulation. (A, B) Representative scatter plots illustrating migration patterns of SCP-1 and SCP-1 dnIKK cells. (C, D) SCP-1 dnIKK cells revealed a significantly increased migration activity towards the TNFα gradient, as shown by a higher center of mass (COM) dislocation and forward migration index (FMI). Compared to a decreased invasion capacity, blocking of IKK-2 resulted in an increased 2-D migration. Data are presented as mean ± SD of three independent experiments. Media control was subtracted in both groups ($^{*}p < 0.05$).

of metalloproteinases [20]. TNFα induced invasion is mediated via the NFkB pathway and strongly depends on IKK-2 activity [9]. Using an ECM coated transwell assay, we demonstrated a significant increase of invasion capacity in SCP-1 cells upon TNFα stimulation. This effect was dramatically reduced by blocking IKK-2 (Fig. 2). We previously demonstrated comparable results in primary hMSC and here show that the immortalized SCP-1 cells feature comparable biological properties in terms of TNFα mediated invasion. Therefore, SCP-1 cells represent a reliable cell model for further investigations in this field. The impaired invasion capacity after blocking IKK-2 might be due to the previously observed downregulation of MMP expression in hMSC [4]. Interestingly, others showed a complete loss of the invasion capacity in hMSC due to a gene knockdown of MMPs [5]. Taken together, impaired MMP expression may represent an underlying factor for the reduced invasion upon TNFα stimulation of SCP-dnIKK cells.

Besides the crucial impact of IKK-2 on invasion, it remains unknown whether blockage of NFkB signalling also leads to alteration or impairment of the cells' migratory response. Only a few reports accurately describe the effect of TNFα on mesenchymal stem cell migration. Zhang and others observed a significant induction of MSC migration upon TNFα stimulation, mainly via TNFRII [11]. The group of Fu et al. demonstrated the dose dependency of this migratory response. The most significant enhancement in migration was found at a concentration of 50 ng/ml and p38 as well as ERK1/2 were phosphorylated upon TNFα stimulation [10]. Our results confirmed an unaltered expression of TNFRI and TNFRII after lentiviral hTERT and dnIKK-2 transduction. Consistent with previous reports, SCP-1 cells showed a significantly enhanced migration upon TNFα stimulation using a concentration of 50 ng/ml on collagen IV coated micro slides (Fig. 3).

Thereafter, we analysed the impact of IKK-2 in terms of TNFα induced 2-D migration. Surprisingly, blocking IKK-2 resulted in a significantly higher migration capacity (Fig. 4). These results are contrary to the changes found in the invasion capacity of SCP1 cells after dnIKK-2 transduction. While invasion capacity strongly depends on a sufficient expression of specific proteolytic enzymes to overcome matrix barriers, directed migration seems to be enhanced once IKK-2 mediated pathways are blocked.

The process of cellular migration begins with cells establishing polarity towards a chemotactic gradient by rearranging the actin cytoskeleton and then extending broad lamellipodia and spike-like filopodia towards the direction of migration. Moreover, migration is described as a very rapid process and typically does not require the synthesis of proteins or proteases for cells to initiate and maintain directed movement [21]. We therefore assume that blocking the energy consuming protease synthesis by dnIKK overexpression may provide larger amounts of energetic equivalents for migratory processes. Other researchers observed increased migration of fibrosarcoma cells after inhibition of pericellular proteolysis. A combination of five pharmacological inhibitors was simultaneously applied to the cells in order to ensure maximum inhibition efficiency by targeting different protease classes known to contribute to ECM degradation. The authors described a transition from proteolytic mesenchymal towards non proteolytic mesenchymal-amoeboid movement. This transition may demonstrate a supramolecular plasticity mechanism and a morphodynamic adaptation in cell migration. The mesenchymal-amoeboid movement was concluded to represent a robust "salvage" strategy of cell movement after the abrogation of pericellular proteolysis [13]. This compensatory mechanism may also underlie the unexpectedly weak benefit of MMP and other proteases inhibitor therapies found

in some animal tumor models [22,23]. These therapeutic approaches aimed to inhibit invasion in terms of metastatic spreading.

The contradictory behavior of dnIKK MSC in terms of 3-D invasion and 2-D migration may also be due to a different impact of cell adhesion molecules in these processes. VCAM-1 and CD44 are known to mediate cell–cell interaction and cell adhesion in mesenchymal stem cells. Our group previously showed that dnIKK overexpression leads to an impaired upregulation of VCAM-1 and CD44 upon TNFα stimulation [4]. The resulting decrease in cell–cell interactions and surface adhesion may contribute to an increase of 2-D migration capacity.

Another explanation for the results in 2-D migration could be a predominant activation of IKK-2 independent pathways, such as such as p38 and ERK1/2. Beside an observed phosphorylation of p38 and ERK1/2 upon TNFα stimulation in MSC, the group of Fu et al. analysed the effect of blocking respective pathways on MSC migration. The authors demonstrated a strong impact of p38, as its inhibition resulted in a significantly reduced migration capacity, whereas, there was no significant effect after blocking ERK 1/2 pathway [10].

Taken together, we here present the contrary effect of IKK-2 blockage on 3-D invasion and 2-D migration in human MSC. While dnIKK-2 transduced MSC reveal a significantly decreased invasion capacity upon TNFα stimulation, there was a significant increase in the migratory response. These finding supports the idea that the TNFα-induced MSC invasion is at least in part mediated by the NFκB pathway, while MSC migration is blocked by this pathway. These contrary effects might cause that MSCs get trapped at the side of tissue injury where typically high TNFα concentration are found. Thus, our study presents *in vitro* data that distinguishes between 3-D invasion and 2-D migration. Both methods, the micro slide chamber and the ECM coated transwell assay, illuminate specific aspects of MSC biological properties that are involved in the complexly orchestrated processes of stem cell recruitment *in vivo*. These findings contribute to a deeper understanding of hMSC recruitment and homing, which is a fundamental part of tissue regeneration. Further studies, however, are needed to elucidate the precise molecular mechanisms directing MSC to the sites of tissue injury.

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References

[1] D. Docheva, D. Padula, C. Popov, W. Mutschler, H. Clausen-Schaumann, M. Schieker, Researching into the cellular shape, volume and elasticity of mesenchymal stem cells, osteoblasts and osteosarcoma cells by atomic force microscopy, J. Cell. Mol. Med. 12 (2008) 537–552.
[2] J.M. Karp, G.S. Leng Teo, Mesenchymal stem cell homing: the devil is in the details, Cell Stem Cell 4 (2009) 206–216.
[3] A. Schmidt, D. Ladage, T. Schinkothe, U. Klausmann, C. Ulrichs, F.J. Klinz, K. Brixius, S. Arnhold, B. Desai, U. Mehlhorn, R.H. Schwinger, P. Staib, K. Addicks, W. Bloch, Basic fibroblast growth factor controls migration in human mesenchymal stem cells, Stem Cells 24 (2006) 1750–1758.
[4] W. Böcker, D. Docheva, W.C. Prall, V. Egea, E. Pappou, O. Rossmann, C. Popov, W. Mutschler, C. Ries, M. Schieker, IKK-2 is required for TNF-alpha-induced invasion and proliferation of human mesenchymal stem cells, J. Mol. Med. (Berl) 86 (2008) 1183–1192.
[5] C. Ries, V. Egea, M. Karow, H. Kolb, M. Jochum, P. Neth, MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines, Blood 109 (2007) 4055–4063.
[6] A.S. Baldwin Jr., The NF-kappa B and I kappa B proteins: new discoveries and insights, Annu. Rev. Immunol. 14 (1996) 649–683.
[7] Z.J. Chen, L. Parent, T. Maniatis, Site-specific phosphorylation of IkappaBalpha by a novel ubiquitination-dependent protein kinase activity, Cell 84 (1996) 853–862.
[8] J.A. DiDonato, F. Mercurio, M. Karin, Phosphorylation of I kappa B alpha precedes but is not sufficient for its dissociation from NF-kappa B, Mol. Cell. Biol. 15 (1995) 1302–1311.
[9] D. Widera, I. Mikenberg, M. Elvers, C. Kaltschmidt, B. Kaltschmidt, Tumor necrosis factor alpha triggers proliferation of adult neural stem cells via IKK/NF-kappaB signaling, BMC Neurosci. 7 (2006) 64.
[10] X. Fu, B. Han, S. Cai, Y. Lei, T. Sun, Z. Sheng, Migration of bone marrow-derived mesenchymal stem cells induced by tumor necrosis factor-alpha and its possible role in wound healing, Wound Repair Regen. 17 (2009) 185–191.
[11] A. Zhang, Y. Wang, Z. Ye, H. Xie, L. Zhou, S. Zheng, Mechanism of TNF-alpha-induced migration and hepatocyte growth factor production in human mesenchymal stem cells, J. Cell. Biochem. 111 (2010) 469–475.
[12] D.A. Lauffenburger, A.F. Horwitz, Cell migration: a physically integrated molecular process, Cell 84 (1996) 359–369.
[13] K. Wolf, I. Mazo, H. Leung, K. Engelke, U.H. von Andrian, E.I. Deryugina, A.Y. Strongin, E.B. Brocker, P. Friedl, Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis, J. Cell Biol. 160 (2003) 267–277.
[14] W. Böcker, Z. Yin, I. Drosse, F. Haasters, O. Rossmann, M. Wierer, C. Popov, M. Locher, W. Mutschler, D. Docheva, M. Schieker, Introducing a single-cell-derived human mesenchymal stem cell line expressing hTERT after lentiviral gene transfer, J. Cell. Mol. Med. 12 (2008) 1347–1359.
[15] W. Böcker, O. Rossmann, D. Docheva, G. Malterer, W. Mutschler, M. Schieker, Quantitative polymerase chain reaction as a reliable method to determine functional lentiviral titer after ex vivo gene transfer in human mesenchymal stem cells, J. Gene Med. 9 (2007) 585–595.
[16] J. Grassinger, D.N. Haylock, M.J. Storan, G.O. Haines, B. Williams, G.A. Whitty, A.R. Vinson, C.L. Be, S. Li, E.S. Sorensen, P.P. Tam, D.T. Denhardt, D. Sheppard, P.F. Choong, S.K. Nilsson, Thrombin-cleaved osteopontin regulates hemopoietic stem and progenitor cell functions through interactions with alpha9beta1 and alpha4beta1 integrins, Blood 114 (2009) 49–59.
[17] C. Lecut, K. Frederix, D.M. Johnson, C. Deroanne, M. Thiry, C. Faccinetto, R. Maree, R.J. Evans, P.G. Volders, V. Bours, C. Oury, P2X1 ion channels promote neutrophil chemotaxis through Rho kinase activation, J. Immunol. 183 (2009) 2801–2809.
[18] G. Broughton 2nd, J.E. Janis, C.E. Attinger, The basic science of wound healing, Plast. Reconstr. Surg. 117 (2006) 12S–34S.
[19] E. Tsiridis, N. Upadhyay, P. Giannoudis, Molecular aspects of fracture healing: which are the important molecules?, Injury 38 (Suppl 1) (2007) S11–25.
[20] H. Yagi, A. Soto-Gutierrez, B. Parekkadan, Y. Kitagawa, R.G. Tompkins, N. Kobayashi, M.L. Yarmush, Mesenchymal stem cells: mechanisms of immunomodulation and homing, Cell Transplant. 19 (2010) 667–679.
[21] M. Penzo, R. Molteni, T. Suda, S. Samaniego, A. Raucci, D.M. Habiel, F. Miller, H.P. Jiang, J. Li, R. Pardi, R. Palumbo, E. Olivotto, R.R. Kew, M.E. Bianchi, K.B. Marcu, Inhibitor of NF-kappa B kinases alpha and beta are both essential for high mobility group box 1-mediated chemotaxis [corrected], J. Immunol. 184 (2010) 4497–4509.
[22] P. Kurschat, P. Zigrino, R. Nischt, K. Breitkopf, P. Steurer, C.E. Klein, T. Krieg, C. Mauch, Tissue inhibitor of matrix metalloproteinase-2 regulates matrix metalloproteinase-2 activation by modulation of membrane-type 1 matrix metalloproteinase activity in high and low invasive melanoma cell lines, J. Biol. Chem. 274 (1999) 21056–21062.
[23] C. Ntayi, S. Lorimier, O. Berthier-Vergnes, W. Hornebeck, P. Bernard, Cumulative influence of matrix metalloproteinase-1 and -2 in the migration of melanoma cells within three-dimensional type I collagen lattices, Exp. Cell Res. 270 (2001) 110–118.