IQGAP1-dysfunction leads to induction of senescence in human vascular smooth muscle cells

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1. Introduction

It is well established that senescent cells can communicate with neighboring cells via secreted factors such as cytokines, chemokines, metalloproteinases and other proteins. This way of communication is commonly known as senescence-associated secretory phenotype (SASP). However, several years ago Biran and colleagues showed that this was not the only possible way of communication between senescent cells (Biran et al., 2015). According to Biran’s findings, senescent cells can also communicate via intercellular protein transfer (IPT). This manner of communication involves direct exchange of proteins between two cells via cellular bridges (CBs) or tunneling nanotubes (TNTs) – tubular protrusions formed between distant cells. Such transfer was first observed and described in immune cells (Gerdes et al., 2007; Önfelt et al., 2004) and rat pheochromocytoma PC12 cells (Rustom et al., 2004). Later, TNTs formation has also been observed in culture for several types of mammalian cells including stem cells, brain derived cells (among them neurons) and cancer cells (Ariazi et al., 2017; Gerdes et al., 2007). TNTs were also reported in vivo, among others, in immune cells residing in lymph nodes (Zaccard et al., 2016) and cancer cells resected from patients with malignant tumors (Ariazi et al., 2017). The intercellular transfer is not limited to proteins and can also include exchange of other cellular components such as vesicles, organelles (e.g. mitochondria) and small molecules. The inducing stimuli and mechanism of TNTs formation are still unclear partially due to the dynamic nature of these structures. However, Biran and colleagues showed that two proteins: actin and Cdc42 were indispensable for the transfer to occur. These authors also proved that the intensity of IPT was increased in senescent cells.

IQGAP1 (IQ motif-containing GTPase activating protein 1) is a scaffold protein involved in the regulation of diverse cellular processes including cytoskeleton remodeling/dynamics. This protein contains several domains and has numerous binding partners, which allows it to coordinate multiple signaling pathways. IQGAP1 was shown to bind actin (via CHD – calponin homology domain) and Cdc42 (via GRD – GAP-related domain), which suggests it could play a role in...
intercellular communication. Despite being involved in such diverse cellular pathways, IQGAP1 was (to our knowledge) never considered in intercellular communication. Despite being involved in such diverse extrinsic factors, such as irradiation or chemotherapeutics, but also intrinsic factors like oxidative stress, telomere erosion or oncogene activation. Several pathways are known to be involved in senescence induction. Activation of these pathways leads not only to proliferation arrest but also causes changes in chromatin structure, gene expression and the profile of secreted proteins. Taking into account that IQGAP1 coordinates some key signaling pathways in the cell, including those involved in proliferation, a connection between IQGAP1 and senescence seems reasonable.

Here, we evaluate the hypothesis that, apart from actin and Cdc42, also IQGAP1 is involved in intercellular communication. To study this issue we used proliferating and senescent human vascular smooth muscle cells (VSMCs). Our results showed that the CBs formed preferentially between senescent cells; however, IQGAP1 was not essential for their generation. Furthermore, we also observed that the introduction of mutated IQGAP1, lacking the GRD or CHD domain, induced senescence in VSMCs.

2. Materials and methods

2.1. Cell culture

Human VMSCs were purchased from ATCC or Lonza and cultured as described before (Bielak-Zmijewska et al., 2014). Cells were treated with curcumin (7.5 μM) or doxorubicin (100 nM) at passages between 7 and 9 (young cells) and after 7 days almost 100 % of cells underwent senescence (validated by an increase in senescence-associated β-galactosidase activity and decreased BrdU incorporation, as described earlier (Bielak-Zmijewska et al., 2014)). Such senescent cells were then used for further experiments. Cells were considered as replicatively senescent when the percentage of senescent cells in the whole cell population exceeded 80 %. For the experiments involving coculture, two differentially stained populations of VSMCs were mixed in equal amounts and seeded.

2.2. Senescent-associated β-galactosidase staining

Detection of senescence-associated β-galactosidase (SA-β-gal) activity was performed according to (Dimri et al., 1995) and as described earlier by us (Bielak-Zmijewska et al., 2014). Cells were analyzed in a light microscope and counted (at least 100 cells).

2.3. BrdU incorporation assay

To assess DNA synthesis the bromodeoxyuridine assay was used (BrdU, Sigma-Aldrich). BrdU was added to the medium (10 μM) and 24 h later cells were detached by trypsinisation (0.25 % Trypsin-EDTA solution, Sigma-Aldrich), centrifuged at 4 °C for 1.5 h in a Beckman Coulter Optima L100 XP ultracentrifuge, in a rotary rotor at 26,000 rpm. The pellet was re-suspended in 1 x PBS, aliquoted and stored at −80 °C. The titer of the produced lentiviral vectors was estimated by real-time PCR (method based on a dsDNA fragment of about 1000 bp present in the lentivector genome within the LTR sequence). The following primers were used: 5′-ACGTTGGCTTAGGTGTTCA-3′ R: 5′-TACTAAAAGGGTCTGAG GGA-3′.

2.5. Transduction and staining with CellTracker Green

For transduction purposes, VSMCs were seeded at a density of about 5 000 cells/cm². Cells were infected in the presence of Polybrene (final concentration 8 μg/mL, Sigma-Aldrich) with pLVmCherry, pLVmCherry-pdCHD or pLVmCherry-pdGRD. After 24 h culture, the medium was changed. Transduction efficacy was determined by the fraction of cells with red fluorescence (mCherry expression) and amounted to (on average) 58 %, 23 % and 45 % for pLVmCherry, pLVmCherry-pdCHD and pLVmCherry-pdGRD, respectively. For staining with CellTracker Green (Thermo Fisher Scientific), VSMCs were seeded at a density of about 3 500 cells/cm² and incubated with the dye diluted 1:5000 in medium without FBS or supplements. After 30 min incubation at 37 °C, cells were washed with PBS twice and full medium was added. Cells were cultured for 24 h before further experiments.

2.6. Cytometry investigation of intercellular communication

After at least 24 h of culture (or co-culture) cells were detached by trypsinisation (0.25 % Trypsin-EDTA solution, Sigma-Aldrich), centrifuged for 5 min at 1000 rpm and resuspended in 400 μl of PBS. Cells were immediately analyzed in FACS Calibur (investigation of intercellular communication between young and senescent VSMCs) or...
LSRFortessa (investigation of the influence of mutated IQGAP1 on intercellular communication) cytometer. For each analyzed 24 h co-culture variant a respective control of two populations of cells mixed just before the measurement was prepared. At least 10,000 cells were analyzed. Experiments were performed at the Laboratory of Cytometry, Nencki Institute of Experimental Biology.

2.7. Time-lapse observation

VSMCs were analyzed with Leica AF7000 Live Imaging System (Leica-Microsystems). Cells were maintained under culture conditions (37 °C, 5% CO₂) and observed for about 3 days. Bright light and red fluorescence images were taken every 6 min. Time-lapses were analyzed by the Fiji software. For non-transduced cells, random cells were analyzed; among transduced cells only VSMCs with red fluorescence were analyzed. The duration of cell division was measured as the time since cells became round until both daughter cells re-attached to the bottom of the well. Experiments were performed at the microscopy core facility in the Laboratory of Imaging Tissue Structure and Function at the Nencki Institute of Experimental Biology.

2.8. Confocal microscopy

VSMCs were stained with CellTracker Green and seeded on glass-
bottom dishes. Live cell imaging was performed using a Leica DMI6000 microscope. The images were overexposed to enable visualization of protrusions and CBs. Experiments were performed at the microscopy core facility in the Laboratory of Imaging Tissue Structure and Function at the Nencki Institute of Experimental Biology.

2.9. Western blot

Whole cell protein extracts were prepared according to Laemmli (Laemmli, 1970). Primary antibodies used were: anti-Cdc42 (1:500), anti-IQGAP1 (1:5000) (Santa Cruz Biotechnology), anti-GAPDH (1:50000) (Millipore). The respective proteins were detected after incubation with appropriate HRP-conjugated secondary antibodies (1:2000) (Dako), using an ECL system (Thermo Fisher Scientific), according to the manufacturer’s instructions.

2.10. Immunofluorescence

For detection of IQGAP1 and actin, VSMCs were grown on cover slides and fixed in 4% PFA. Cells were stained as described before by us (Grabowska et al., 2016). The primary antibody used was: anti-IQGAP1 (1:500) (Santa Cruz Biotechnology); followed by secondary antibody conjugated with Alexa Fluor 488 (Thermo Fisher Scientific). Phalloidin (1:500) (Sigma-Aldrich) was used for actin visualization.

2.11. Measurements of secreted factors

Secreted factors were measured in culture medium conditioned for 24 h. Secretory phenotype was analyzed by Cytometric Bead Array Human Soluble Protein Master Kit (Becton Dickinson) according to manufacturer’s protocol. The measurement was made using a BD LSRFortessa cytometer equipped with BD FACSDiva 6 software. Results were analyzed with FCAP Array™ 3.0 software.

2.12. Statistical analysis

The normality was tested with the Shapiro-Wilk test and then an appropriate statistical test was used. Kruskal-Wallis test was used to analyze the level of IQGAP1 and Cdc42, intercellular communication between young and senescent VSMCs, BrdU incorporation, time of division and the activity of SA-β-gal. One-way Anova was used to analyze influence of mutated IQGAP1 on intercellular transfer, proliferation and the level of cytokines. Data are presented as a mean ± SEM. A value of p < 0.05 was considered statistically significant (ns – no significance, p < 0.05-*, p < 0.01-**, p < 0.001-***). GraphPad Prism 8 software was used for calculations. All graphs show the mean results from at
least 3 independent experiments.

3. Results

3.1. Localization and expression of IQGAP1 in young and senescent VSMCs

Two types of senescence were analyzed, namely replicative and premature ones. In VSMCs at early passages endogenous IQGAP1 is uniformly distributed across the cytoplasm and is organized in a tight net (Fig. 1A). After induction of senescence, IQGAP1 becomes more dispersed and the net is less dense. This is more prominent in VSMCs which underwent replicative senescence than in those undergoing stress-induced premature senescence (SIPS) upon treatment with doxorubicin or a cytostatic dose of curcumin. In all analyzed variants – young and senescent cells – IQGAP1 is present in CBs/TNTs and other protrusions formed by VSMCs (Fig. 1A) where it co-localizes with actin (Fig. 1B). Such co-localization could suggest that IQGAP1 potentially participates in CBs formation and hence in intercellular communication. The level of endogenous IQGAP1 decreased in replicatively senescing VSMCs in vitro (Fig. 1C and D) and was reduced by 30 % already at passages 15–22. A similar tendency was observed in cells undergoing SIPS. Interestingly, a decrease in IQGAP1 level was observed already 1 day after doxorubicin/curcumin treatment, which can be associated with proliferation arrest, and was sustained due to senescence induction. Moreover, we observed a gradual decrease in the level of endogenous Cdc42 in cells undergoing RS and SIPS (Fig. 1C and E). After 7 days of doxorubicin or curcumin treatment, only half of the initial amount of this protein was observed.

The localization and the level of IQGAP1 in VSMCs were similar after doxorubicin and curcumin treatment, however, we decided to use doxorubicin for SIPS induction in the subsequent experiments as curcumin is a lipophilic agent, which can localize in membranes and could interfere with protrusion formation.

3.2. Intercellular communication between young and senescent VSMCs

To analyze the efficiency of intercellular communication in VSMCs we assessed the transfer of the fluorescent mCherry protein or of the CellTracker Green dye using flow cytometry. The results of transfer after 24 h of co-culture (24 h; Fig. 2A) was compared to the results obtained for cells mixed in a test tube just before the measurement (0 h; Fig. 2A). We investigated the intercellular transfer in 4 experimental combinations that is between: (C + C) differentially stained populations of young cells, (C + SIPS) young cells (green) and cells undergoing SIPS (red), (C + RS) young cells (green) and replicatively senescent VSMCs (red), and (RS + SIPS) replicatively senescent VSMCs (green) and cells undergoing SIPS (red). We distinguished 4 populations of cells: not stained (ns) (in R3 region), red (R4 region), green (R2 region) and cells which stained with either the red or the green dye (regions R5 and R6). Percentage of cells localizing in regions R5 and R6 in variants C + C 0 h, C + SIPS 0 h and C + RS 0 h was comparable to the data obtained for not stained cells (background level) suggesting that no exchange of dye occurred. In all the analyzed variants we observed that after 24 h of co-culture the population of ns and red cells moved up the Y axis, which suggests that cells received the green dye, while the population of green cells moved right along the X axis indicating that they took in mCherry (Fig. 2A). The greatest transition was observed for the co-culture of two populations of senescent cells (RS + SIPS), where almost 25 % of cells received the dye (Fig. 2B), however, this result was not statistically significant. Curiously, statistically significant exchange after 24 h of co-culture was observed in the remaining variants, even though the rate was lower. It was especially visible in the C + RS variant, where less than 10 % of cells transferred a dye. Objective evaluation of the exchange rate (exchange after 24 h reduced by the exchange at 0 h) showed that there were no statistically significant differences in the exchange rate between different analyzed variants (Fig. 2C).
3.3. Influence of mutated IQGAP1 on intercellular communication and protrusion formation

To evaluate the role of IQGAP1 in protrusion formation and the intercellular transfer we generated cells expressing mutated forms of the protein: one lacking the CHD domain responsible for actin binding, and the other lacking the GRD domain, which has the ability to bind Cdc42 (Fig. 3A). We did not observe any significant differences in dye exchange and the ability to form CBs in cells in which mutated forms of IQGAP1 were expressed (independently of whether they lacked the GRD or CHD domain) in comparison to wild type cells (Fig. 3B, C).

Even though the deletion of the individual domains had no impact on the intracellular communication and CBs formation, we observed that expression of either form of mutated IQGAP1 caused alterations in VSMCs' morphology. Cells increased their size, became more flat and less cells were visible in a field of view, which suggests diminished proliferation potential (Fig. 4A). No spectacular changes in the distribution of IQGAP1 were visible (Fig. 4B); however, we noticed the formation of stress fibers in VSMCs expressing either form of mutated IQGAP1 (Fig. 4C). All the above mentioned morphological features of VSMCs expressing mutated IQGAP1 resembled those observed in senescent cells.

We decided to investigate if such spectacular morphological changes in VSMCs expressing mutated IQGAP1 were followed by alterations in the proliferation rate and physiology. We observed that the cell number decreased by almost 40% (Fig. 5A), which was further substantiated by a drop in the number of cells incorporating BrdU when IQGAP1 was mutated (Fig. 5B). Among VSMCs expressing IQGAPΔCHD
and IQGAPΔGRD 39 % and 34.5 % of cells, respectively, incorporated BrdU in comparison to control cells (transfected with mCherry only) for which the percentage was 62 %. This suggests that lack of GRD or CHD domain leads to a decrease in DNA replication and proliferation in VSMCs. Time-lapse analysis revealed that VSMCs with mutated IQGAP1 needed more time to complete cell division (Fig. 5C). VSMCs expressing IQGAP1ΔCHD needed, on average, 7 extra minutes (14 % more time) to complete division while, when IQGAP1ΔGRD was expressed, the division time was 9 min (19 %) longer than in control cells (expressing only mCherry) the division of which lasted about 52 min. Moreover, we observed that among VSMCs expressing mutated IQGAP1 less cells entered mitosis. Some of the cells which began the division (detached, rounded up and attempted to form a mitotic spindle) failed to complete the process and either stuck to the bottom of the dish again or died (full detachment and/or fragmentation) (see Supplementary materials Sup.movies 1–4).

3.4. Lack of wild type IQGAP1 leads to senescence of VSMCs

The senescent morphology, decreased proliferation and prolonged mitosis, encouraged us to test if deletion of GRD or CHD domain of IQGAP1 leads to senescence of VSMCs. Indeed, we observed an increased number of cells with elevated activity of senescence-associated β-galactosidase (SA-β-gal) – a most commonly used senescence marker (Fig. 6A,B). The effect was most prominent in VSMCs expressing IQGAP1ΔGRD of which 68 % were SA-β-gal positive, and a little less pronounced when IQGAP1ΔCHD was present – 52 % of positive cells in comparison to 25 % in control and 37 % in VSMCs expressing mCherry. Cells expressing mutated IQGAP1 were also characterized by increased secretion of pro-inflammatory cytokines – interleukins IL1β, IL6 and IL8, the components of SASP (Fig. 6C). The most significant increase was observed for IL6 as cells with IQGAP1ΔGRD secreted on average 10.5 times more, and those with IQGAP1ΔCHD 11 times more, of this cytokine than control cells. These cells also secreted 5 (IQGAP1ΔGRD) and 3.7 (IQGAP1ΔCHD) times more of IL1β. The effect was the least pronounced on IL8 secretion as cells with each mutated IQGAP1 secreted 1.4 times more of this interleukin in comparison to wild type cells. An increase was also observed for cells expressing mCherry alone, but only in the case of IL6 and IL1β. It amounted to 4 and 2 times higher secretion than in control, respectively, which was much less than in cells with mutated IQGAP1.

4. Discussion

Transfer of cellular components via TNTs was discovered relatively recently. It was first described in 2004 by Rustom (Rustom et al., 2004) in PC12 rat cell line. Since then TNTs were observed in a variety of cell lines and in vivo; however, the mechanism of TNTs formation and function is still elusive. TNTs are rarely observed under normal conditions (Ariazi et al., 2017); however, TNT-mediated communication becomes much higher under pathological conditions such as a viral or bacterial infection, cancer, prion diseases or synucleino- and tauopathies (Ariazi et al., 2017). TNTs can facilitate the spread of infection or toxic agents. More insights in the structure and properties of TNTs...
will let us better understand the role of this form of communication. It is especially important in the light of recent findings of Biran and colleagues that such form of cell-to-cell communication is very important in senescence. Senescent cells undergoing both replicative and stress-induced senescence exhibit enhanced TNTs formation and intercellular transfer (Biran et al., 2015). Increased formation of protrusions was also observed in H2O2-treated astrocytes (Zhu et al., 2005). This suggests that the frequency of this kind of communication can increase with age. Such an increase has two effects – on the one hand it allows cells to postpone senescence. It was shown that TNT-mediated transport has a restorative role in mesenchymal stem cells (MSCs) spheroids (Whitehead et al., 2020). Senescent MSCs can transfer the cell cycle inhibitor, p16, to cells of early passages, which reduces the effect of senescence in the former cells and allow them to maintain regenerative properties. Other authors have shown that TNT-mediated exchange of the lysosomal pool can rescue prematurely senescent endothelial cells (Yasuda et al., 2011). On the other hand, however, when senescing cells try to rescue themselves by getting rid of unwanted molecules, cells which are on the receiving end of this arrangement acquire deleterious factors, including cell cycle inhibitors, damaged mitochondria or misfolded protein aggregates, such as those causing prion diseases and tauopathies (Tardivel et al., 2016), which can contribute to disease spread. Despite all the data supporting the rise in TNT-mediated transport in senescent cells, our results suggest that the increase in intercellular communication during senescence is not a universal feature. We did observe significant dye exchange between differentially colored VSMCs after 24 h of co-culture in almost all experimental groups, however, the rate of exchange was similar regardless of the senescence status of the cells. The lowest transfer was observed for the co-culture of proliferating and senescent VSMCs (not statistically significant). This can be related to the change in properties and composition of the cell membrane, which occurs during cellular senescence. This applies not only to lipid composition but also membrane receptors (reviewed in (Fulop et al., 2012)), which are responsible for intercellular communication and can be involved in TNTs formation. Similarity of cell membrane composition could favor membrane fusion and TNTs formation. Moreover, senescent cells are bigger, which increases the surface area available for formation of protrusions.

Interestingly, we observed increased exchange of dyes between two populations of senescent cells already at 0 h (RS + SIPS 0 h). Biran et al. in their work from 2014 described that direct intercellular transport can occur very rapidly (within seconds). However, such effect can be also caused by sticking of the cell fragments to the cell membrane. We observed that senescent VSMCs often leave behind small fragments of the cytoplasm from the rear edge of the cell as a result of migration (time-lapse observation, not published), which can stick to the outer membrane of other cells. Such effect was not observed during culture of young VSMCs. Biran and colleagues showed that increase in the intercellular communication observed during senescence is associated with increased protein, but not mRNA, level of Cdc42, which, along with actin, was found crucial for the intercellular transfer to occur. Other authors have shown that the level of Cdc42 increases during senescence of hematopoietic stem cells (HSCs) and that inhibition of Cdc42 rejuvenated these cells (Florian et al., 2012). We, on the contrary, show that in senescent VSMCs the level of Cdc42 is decreased, which suggests that this effect is cell-specific or stimuli-specific. Biran’s studies were conducted on human fibroblasts. Moreover, senescence was induced by etoposide treatment or overexpression of Ras oncogene. It was shown that Cdc42 is essential for Ras-induced transformation (Stengel and Zheng, 2012), which can explain the increase in Cdc42 level.

Our hypothesis presumed that, apart from actin and Cdc42, also IQGAP1 is involved in intercellular communication and TNTs formation. It is well established that IQGAP1 mediates Cdc42 and actin interaction during cytoskeleton remodeling (reviewed in (Abel et al., 2015)). We have observed that IQGAP1 is present in TNTs and cellular protrusions. However, our results do not support the hypothesis that this protein is indispensable for CBs formation and cell-to-cell communication.

Nevertheless, we obtained unexpected and interesting results. Expression of mutated IQGAP1 lacking the GRD or CHD domain induced senescence in VSMCs. We observed a decrease in proliferation, increased cell division time, senescence-specific changes in morphology and induction of some senescence markers, namely SASP, stress fibers and increased activity of SA-β-gal. The abovementioned features contribute to the senescence phenotype of VSMCs, as described by us earlier for cells undergoing replicative senescence or treated with DNA damage inducing (doxorubicin) or other stress inducing agents (curcumin, H2O2) (Bielak-Zmijewska et al., 2014; Grabowska et al., 2016, 2015; Przybylska et al., 2016). Some of the senescence markers listed above were less pronounced in cells with mutated IQGAP1 comparing to what we observed in our previous works. However, we need to take into account that not all of the analyzed VSMCs expressed mutated IQGAP1 due to transcription efficacy lower than 100 %. IQGAP1 is believed to be the most complicated scaffold protein with more than 90 known interacting partners (reviewed in (Hedman et al., 2015), and it orchestrates multiple cellular processes. Thus, it is hard to speculate, which of its diverse functions is disrupted when the CHD/GRD domain is missing and which perturbation is directly responsible for the induction of senescence.

The role of IQGAP1 in regulation of cell proliferation has already been reported. For example it was demonstrated that IQGAP1 induced proliferation of VSMCs as a response to estrogen in the development of varicosity (Huang et al., 2014). There are also data showing that IQGAP1 promotes proliferation in different types of normal cells, e.g. in bronchial epithelial cells after injury (Wang et al., 2008a, 2008b), endothelial cells (Meyer et al., 2008), but also in tumor cells (reviewed by White (White et al., 2009)). It is documented that in tumor cells the expression of IQGAP1 is elevated. There are several players involved in the modulation of proliferation, such as mTor, PCNA and β-catenin/Tcf signaling, which were shown to interact with IQGAP1 (Wang et al., 2008a) or the effect can be related to an elevated level of phosphorylated Akt and ERK (Huang et al., 2014). Others suggested that the impact on cell proliferation can be a result of the cross-talk of IQGAP1 with Cdc42-mTor signaling (Wang et al., 2009).

Which of abovementioned pathways (if any) is responsible for modulation of VSMC proliferation when the GRD/CHD domain is missing can only be speculated based on already published data. IQGAP1 is involved in cytokinesis and localizes in the contractile ring during cell division (Bielak-Zmijewska et al., 2008). However, contrary to Wang et al. (Wang et al., 2009), we did not observe impaired cytokinesis. Authors of that study have shown that the impact of IQGAP1 on proliferation is domain-dependent and requires the interaction of IQGAP1 with Cdc42. Another mechanism which may be involved in the regulation of cell proliferation by IQGAP1 can be connected with its nuclear function/localization. IQGAP1 participates in the progression of the cell cycle. It translocates to the nucleus during early S phase (Johnson et al., 2011) and its accumulation was observed in the nuclei of cells arrested in the G1/S phase. Authors of that paper observed delayed progression from the S to G2/M phase in cells with silenced IQGAP1. Our data has shown that in the presence of mutated IQGAP1 the time needed to execute mitosis increased; thus, it seems that a different mechanism is involved in our model. Our results revealed a reduced proliferation potential when Cdc42-binding but also the actin-binding domain were lacking. Until now, the impact on cell proliferation was only observed when the Cdc42-binding domain was missing, which can be explained by the influence of IQGAP1 on Cdc42-mTor signaling. Others have shown that when the GRD domain was missing cells became rounded and substantially reduced their size (Swaat-Mataraza et al., 2002), what was not observed by us. The same authors observed that lack of the GRD domain caused microspike reduction (Swaat-Mataraza et al., 2002). Other studies demonstrated that,
depending on the site of IQGAP1 mutation, the C- (containing CHD domain) or N-terminus (containing GRD domain), the cell size decreased (mutation in the C-terminal part) or increased (when the N-terminal part was missing). This effect can be explained by the interaction with mTor, which is strictly linked to cell proliferation (Wang et al., 2009). It was shown that IQGAP1 promotes the switch from a contractile to a synthetic phenotype of VSMCs (Huang et al., 2014), which in part can be responsible for the increase in proliferation (synthetic VSMCs are characterized by higher proliferation rate). However, it was also shown that in vitro culture, most of the VSMCs attain the synthetic phenotype (Davis-Dusenbery et al., 2011).

Another interesting question is why cells expressing mutated IQGAP1 attained senescence. No one has yet reported any connection of IQGAP1 with senescence. As the induction of the senescent phenotype was evoked by lack of the CHD or GRD domain, we can suspect that one (or more) of the proteins interacting with one (or both) of those domains are responsible for such effect. It seems that lack of the GRD domain was associated with stronger phenotyping effect. There is not much information available in the literature on the influence of the mutation of said domains on the cell phenotype. It was shown that lack of the GRD and, to a lesser extent, of the CHD domain, is associated with changes in IQGAP1 localization pattern in the leading edge and retracting area in mice melanoma cells (Reimer et al., 2017). However, the authors did not describe any effect on morphology, migration or proliferation in the investigated cells. One of the possible scenarios is that cellular stress can be responsible for senescence induction. It is well known that senescence can be a response to stress conditions. IQGAP1 is an essential protein in many crucial cellular processes therefore its mutations may influence multiple signaling pathways and can be recognized as a signal to limit proliferation.

5. Conclusions

IQGAP1 is not essential for the direct transfer occurring preferentially between senescent cells. Moreover, we show for the first time that IQGAP1 can be involved in protection from senescence, as mutated IQGAP1, lacking the GRD or CHD domain, induces senescence in VSMCs.

Declaration of Competing Interest

None.

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The manuscript does not contain clinical studies or patient data.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.molimm.2020.112205.

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