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Regulation of cellular senescence by retinoid X receptors and their partners

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ABSTRACT

Cellular senescence is a response characterized by a stable cell proliferation arrest and a senescence-associated secretory phenotype (SASP) which can be induced by many stresses, including telomere shortening and oncogene activation. Senescence is crucially involved in a variety of physiopathological contexts, such as cancer and aging. Given the fundamental role of this process, senescence needs to be tightly regulated. In the last decade, the key implication of nuclear receptors in cellular senescence has emerged. Here we will review the mechanisms involved in the control of cellular senescence by retinoid X receptors (RXRs) and their partners. We will also present our current knowledge on the regulation of these receptors during senescence and on their potential role in senescence-associated physiopathological conditions.

1. Introduction

Cellular senescence can be induced by a wide variety of stresses such as telomere shortening, oncogene activation, and oxidative and genotoxic stresses. Senescent cells are characterized by two main features: a stable cell cycle arrest and a senescence-associated secretory phenotype (SASP) containing a plethora of proteins including cytokines and chemokines (usually pro-inflammatory), growth factors and proteases (Bernard, 2018; Kuilman et al., 2010) (Fig. 1). This secretome can reinforce senescence in a cell-autonomous (autocrine) manner, induce it and reinforce it in a non-cell-autonomous (paracrine) manner, and exert other paracrine effects on neighboring cells. Senescent cells also display several additional changes, in their morphology, physiology and chromatin organization. These cells are frequently flattened and enlarged. They exhibit a senescence-associated β-galactosidase activity (SA-β-gal) which is experimentally used as a marker to detect them. An accumulation of reactive oxygen species (ROS) is also often observed in senescent cells, induced by alterations in mitochondria and triggering DNA damage. In addition, senescence-associated heterochromatin foci (SAHF) repressing the expression of genes which promote cell cycle progression are formed in some contexts (Hernandez-Segura et al., 2018).

Cellular senescence is now recognized as a key cellular process playing a crucial role in many physiopathological conditions (He and Sharpless, 2017) (Fig. 1). Senescence is induced throughout life, at various stages and in different contexts, and can have either beneficial or detrimental effects on the organism. Senescence contributes to normal embryonic development and promotes wound healing, cell plasticity, stemness and tissue regeneration. It also acts as a major tumor suppressive barrier in the first steps of tumorigenesis (tumor initiation). Indeed, in response to oncogenic signals, oncogene-induced senescence (OIS) is activated and through the SASP triggers the immune clearance of cells at risk of transformation. However, accumulation of senescent cells with time has been shown in the last few years to promote organismal aging and many aging-associated pathologies such as cataract, type II diabetes, lung degenerative disorders, cardiac aging, glomerulosclerosis, atherosclerosis, osteoarthritis, neurodegenerative diseases as well as cancer relapse and progression (Bussian et al., 2018; Childs et al., 2017; Perez-Mancera et al., 2014).

Given its critical impact *in vivo*, cellular senescence needs to be tightly regulated, both spatially and temporally (Hernandez-Segura et al., 2018; Salama et al., 2014) (Fig. 1). The stable cell cycle arrest is mainly implemented by the p53 and RB (retinoblastoma) tumor suppressors. In response to stresses p53 is stabilized and activated through the ARF/MDM2 axis and/or through the ATR-CHK1 and ATM-CHK2 DNA damage response pathways. The expression of CDKN1A encoding the cyclin-dependent kinase inhibitor p21 is induced by p53 and the expression of CDKN2A, encoding the cyclin-dependent kinase inhibitor p16, can increase as well. RB, the phosphorylation of which is inhibited by p21 and p16, represses the expression of E2F target genes promoting cell cycle progression such as cyclins. The SASP is controlled both at the level of transcription, especially by the NF-κB and CEBP-β transcription factors and by the NOTCH signaling pathway, and at the level of translation by the mTOR pathway (Bernard, 2018; Hernandez-Segura

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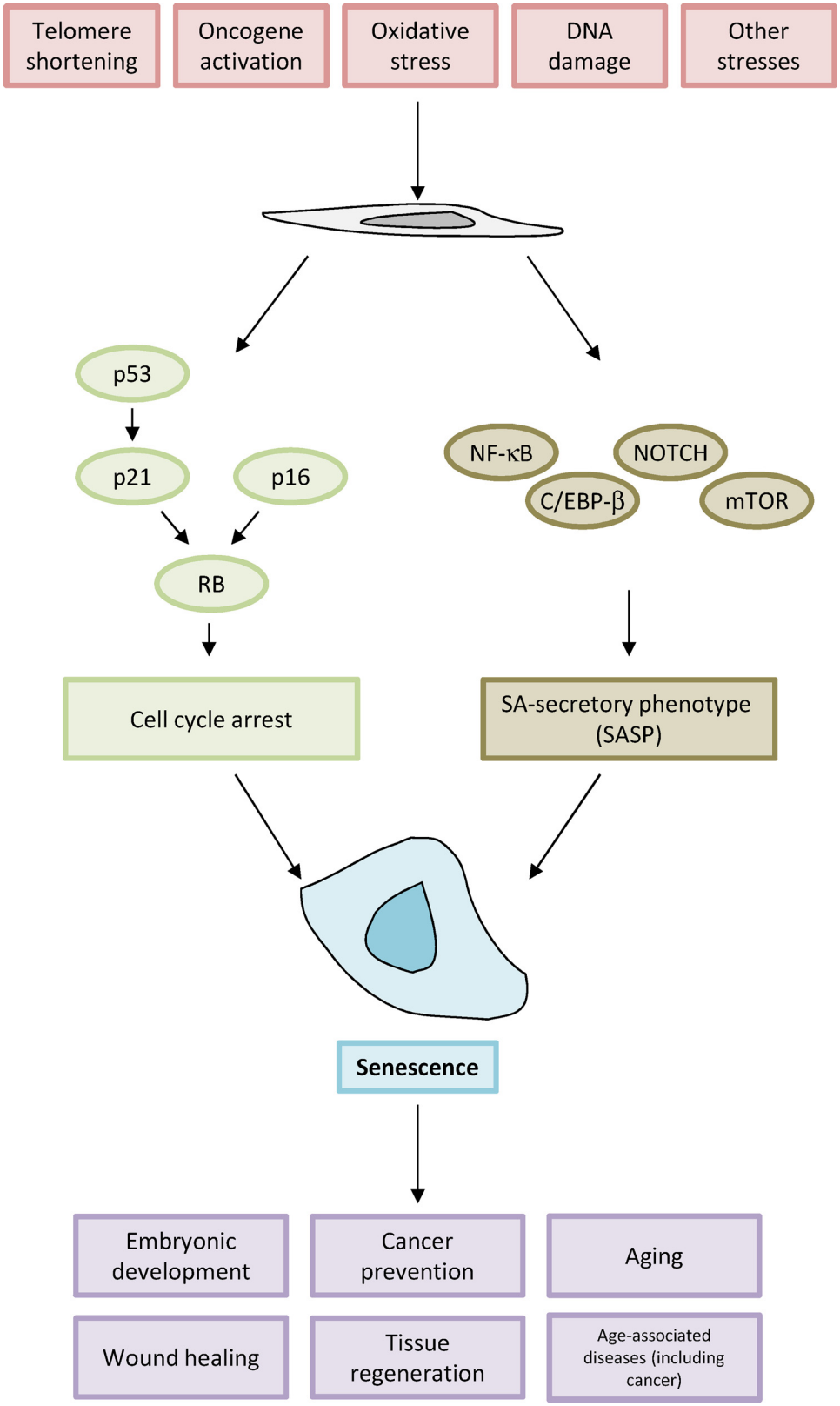

Fig. 1. Overview of cellular senescence. Many different stresses induce effector pathways implementing cellular senescence. Only the main pathways leading to the establishment of the main senescence features, cell cycle arrest and senescence-associated secretory phenotype (SASP), are depicted here. Cellular senescence then plays a crucial role in a wide variety of physiopathological contexts.

et al., 2018; Kuilman et al., 2010). Beyond these elements, factors and mechanisms regulating senescence are still largely unknown and significant efforts have recently been made to identify them. In the last decade, there has been accumulating evidence in the literature for a key role of nuclear receptors in cellular senescence. In this review we will present and discuss the implication of retinoid X receptors (RXRs) and their partners in cellular senescence.

2. Retinoid X receptors

Nuclear receptors, to which RXRs belong, form a superfamily of phylogenetically-related proteins comprising 48 members in humans. Nuclear receptors mainly function as transcription factors and represent one of the largest groups of transcriptional regulators. They share a common structural organization comprising a DNA-binding domain (DBD), a ligand-binding domain (LBD) and some transactivation regions and act as monomers, homodimers or heterodimers. In the absence of ligand, nuclear receptors are either unable to interact with DNA or can function as transcriptional activators or repressors depending on the receptor, context and target genes. Ligands of nuclear receptors, which include steroid hormones, retinoic acids, thyroid hormones, fatty acids, leukotrienes and prostaglandins, regulate their transcriptional activity. However, for a subset of receptors called orphan nuclear receptors, no natural ligand has been identified so far (Evans and Mangelsdorf, 2014; Sever and Glass, 2013).

Nuclear receptors are classified into four classes depending on their mode of action. Type I nuclear receptors reside in the cytoplasm in the absence of ligand, and ligand binding triggers their release from chaperones, their dimerization and their entry into the nucleus where they induce gene expression by association with co-activators. Type II nuclear receptors are located in the nucleus bound to their DNA target sequences. In the absence of ligand, they act as transcriptional repressors through interaction with NcoR and SMRT corepressor complexes associated with histone deacetylases. Ligand binding provokes the dissociation of corepressors, the recruitment of coactivators displaying histone acetyltransferase activity such as PCAF and p300 and the subsequent induction of gene expression (Sever and Glass, 2013). Type III nuclear receptors, functioning as variants of type I, and type IV nuclear receptors, which bind DNA as monomers, have also been identified but are currently not well characterized (Evans and Mangelsdorf, 2014; Sever and Glass, 2013).

Three highly conserved retinoid X receptors (RXRs) are known in humans (RXRα, RXRβ, RXRγ). They are receptors for 9-cis retinoic acid and they each function both as homodimers or heterodimers. RXRs belong to type II nuclear receptors and are central members of this group as the other type II nuclear receptors generally form heterodimers with them. Partners of RXRs include liver X receptors (LXRα, LXRβ), peroxisome proliferator-activated receptors (PPARα, PPARβ/δ, PPARγ), retinoic acid receptors (RARα, RARβ, RARγ), thyroid hormone receptors (TRα, TRβ) and vitamin D receptor (VDR) (Evans and Mangelsdorf, 2014) (Table 1).

Nuclear receptors play key roles *in vivo*, being implicated in organismal development, homeostasis, metabolism and reproduction. At the cellular level they control important cellular processes such as cell differentiation, cell death and lipid metabolism, which are critical for diseases such as cancer, osteoporosis and diabetes (Evans and Mangelsdorf, 2014; Sever and Glass, 2013). We have recently identified senescence as a cellular process regulated by RXRα (Ma et al., 2018). In the next sections we will review the mechanisms involved in the control of cellular senescence by RXRs and their partners, discussing the findings of our laboratory and others. We will also present the current knowledge on the regulation of these receptors during senescence and on their role in senescence-associated physiopathological conditions.

3. Regulation of senescence by retinoid X receptors

Calcium signaling has recently emerged as a new mechanism controlling cellular senescence (Martin and Bernard, 2018). In a senescence bypass screen performed several years ago based on a whole genome shRNA library, our laboratory identified the inositol 1, 4, 5-trisphosphate receptor type 2 (ITPR2) (Vervloessem et al., 2015) as crucial in the implementation of cellular senescence (Wiel et al., 2014). IP3 receptors (ITPR1, ITPR2 and ITPR3) are endoplasmic reticulum (ER) calcium release channels. Their activity is known to be controlled by post-translational modifications (such as phosphorylation) and protein interactions (for example with the BCL2 anti-apoptotic protein) (Akl and Bultynck, 2013). However, little is known about their regulation at the expression level.

In order to identify regulators of ITPR2 expression, we recently performed a screen using a siRNA library targeting 160 epigenetic regulators and transcription factors. Interestingly the nuclear receptor RXRα was identified by this approach as able to repress ITPR2 expression (Ma et al., 2018). Since RXRα had never been reported to impact calcium signaling and cellular senescence we focused on this regulator (Evans and Mangelsdorf, 2014; Szanto et al., 2004). We demonstrated that RXRα is able to bind to the ITPR2 DNA sequence, which was also corroborated upon interrogation of the ENCODE ChIP-seq database. RXRα was thereby characterized as a direct repressor of ITPR2. We further showed that RXRA knockdown in primary human fibroblasts induces the release of calcium from the ER through ITPR2 and its accumulation in mitochondria through the mitochondrial calcium uniporter MCU, which, interestingly, was also identified as a senescence regulator together with ITPR2 in our senescence bypass screen (Ma et al., 2018). Increase in mitochondrial calcium concentration triggered by RXRA knockdown leads to ROS production, DNA damage, p53-dependent induction of CDKN1A expression and finally to cellular

Table 1
Retinoid X receptors and their main partners.

Abbreviation	Name	Nomenclature	Ligand
LXRα	Liver X receptor α	NR1H3	Oxysterols
LXRβ	Liver X receptor β	NR1H2	Oxysterols
PPARα	Peroxisome proliferator-activated receptor α	NR1C1	Fatty acids
PPARβ/δ	Peroxisome proliferator-activated receptor β/δ	NR1C2	Fatty acids
PPARγ	Peroxisome proliferator-activated receptor γ	NR1C3	Fatty acids
RARα	Retinoic acid receptor α	NR1B1	Retinoic acids
RARβ	Retinoic acid receptor β	NR1B2	Retinoic acids
RARγ	Retinoic acid receptor γ	NR1B3	Retinoic acids
RXRα	Retinoid X receptor α	NR2B1	9-cis retinoic acid
RXRβ	Retinoid X receptor β	NR2B2	9-cis retinoic acid
RXRγ	Retinoid X receptor γ	NR2B3	9-cis retinoic acid
TRα	Thyroid hormone receptor α	NR1A1	Thyroid hormones
TRβ	Thyroid hormone receptor β	NR1A2	Thyroid hormones
VDR	Vitamin D receptor	NR1I1	1α, 25-dihydroxyvitamin D_3

senescence. Both a p53/p21-dependent cell proliferation arrest and a senescence-associated secretory phenotype (SASP) were observed upon RXRA knockdown. Conversely, we showed that RXRα overexpression was able to repress this signaling cascade and to delay replicative senescence of primary human fibroblasts. This work was the first to demonstrate that RXRα controls cellular senescence and that this effect depends on RXRα-induced regulation of calcium signaling (Ma et al., 2018).

A rise in intracellular calcium levels and a key role for this calcium accumulation in the implementation of cellular senescence were reported in response to many senescence-inducing stresses including telomere shortening, oncogene activation and oxidative stress (Martin and Bernard, 2018). Mitochondrial dysfunction and ROS production, which are induced by elevated calcium concentration in the mitochondria, can be observed in multiple senescence contexts and were established as major triggers of cellular senescence, activating the DNA damage/p53/CDKN1A cascade (Lee et al., 1999; Passos et al., 2007; Acosta et al., 2008) as observed with RXRA knockdown (Ma et al., 2018). Interestingly, in the last few years, the SASP was shown to be also closely regulated by mitochondria and ROS, which activate the nuclear factor-κB (NF-κB) (Correia-Melo et al., 2019; Nelson et al., 2018). This could be a way by which RXRA knockdown induces the SASP (Ma et al., 2018).

In accordance with our findings, retinoids were reported to be able to activate CDKN1A expression (Liu et al., 1996). Moreover, ligand-activated RXRα and RXRA knockdown were shown to trigger a cell cycle arrest in G1 in cancer cells, associated with an increased expression of CDKN1A and a decreased expression of genes promoting cell cycle progression such as cyclin D1, cyclin E and the proliferating cell nuclear antigen PCNA. CDKN1A upregulation and PCNA downregulation were due to the inhibition of the NF-κB pathway by RXRA knockdown in this context, and cyclin D1 downregulation to the inhibition of the Wnt/β-catenin pathway (Huang et al., 2015). RXRα can thus control CDKN1A expression both indirectly by regulating the ITPR-calcium-ROS-DNA damage-p53 cascade (Ma et al., 2018) or the NF-κB pathway (Huang et al., 2015) and directly by binding retinoid X response elements in the promoter of this gene (Tanaka et al., 2007). Interestingly, a positive feedback loop, where long-term activation of CDKN1A causes mitochondrial dysfunction and generation of ROS, was shown to contribute to the establishment of cellular senescence (Passos et al., 2010). This mechanism could participate in the implementation and maintenance of cellular senescence upon RXRA knockdown (Ma et al., 2018).

In order to gain further insight into the regulation of calcium signaling by RXRα we analyzed the effect of RXRA knockdown on the expression of the two other IP3 receptors, which also function as ER calcium release channels. Interestingly, although no variation in ITPR3 expression was observed, ITPR1 expression was induced as well as ITPR2 expression by RXRA knockdown (Fig. 2A). This observation thus raises the possibility that RXRα controls calcium signaling and cellular senescence not only through ITPR2 but also through ITPR1.

As there are three RXR receptors (RXRα, RXRβ, RXRγ), which are highly conserved and share the same 9-cis retinoic acid ligand and heterodimerizing partners (Evans and Mangelsdorf, 2014), we next wondered whether other RXR than RXRα could also regulate calcium signaling and cellular senescence. We observed that RXRB knockdown induces the expression of ITPR1, ITPR2 and the p53 target genes CDKN1A and GDF15 (Fig. 2B) and triggers premature senescence in primary human fibroblasts (Fig. 2C–D) similarly to the knockdown of RXRA. These data suggest that the ability to control calcium signaling and cellular senescence might be shared by RXR receptors. However, the possible involvement of RXRγ in cellular senescence remains to be investigated, as well as the relevance of these observations in different contexts of senescence, *in vitro* and *in vivo*.

4. Regulation of senescence by partners of RXRs

As mentioned above, other type II nuclear receptors commonly bind DNA following their heterodimerization with RXR receptors. We assessed whether some of these receptors also regulate calcium signaling and cellular senescence. We found that knockdown of PPARG and THRA induces the expression of ITPR1, ITPR2 and the p53 target genes CDKN1A and GDF15 (Fig. 2B) and provokes premature senescence in primary human fibroblasts (Fig. 2C–D). These data suggest that several type II nuclear receptors could potentially regulate cellular senescence by controlling ITPR1/2 expression and calcium signaling (Fig. 3).

PPARγ has already been described to control cellular senescence. Treatment of bladder cancer cells with an agonist of PPARγ induced the expression of CDKN1A and CDKN2A, alongside a reduced cyclin D1 expression and a cell cycle arrest in G1 (Guan et al., 1999). In human normal lung fibroblast strains 2BS and WI38, PPARγ was shown to bind to a peroxisome proliferator response element (PPRE) in the CDKN2A promoter and ligand-induced activation of PPARγ was found to promote cellular senescence though CDKN2A induction. PPARγ silencing suppressed senescence features in these strains, which contrasts with our own data in MRC5 primary lung fibroblasts (Fig. 2B–D), and PPARγ overexpression in untreated cells inhibited their proliferation, concomitantly to CDKN2A regulation (Gan et al., 2008). Adding more complexity to the scene, PPARγ overexpression in human diploid fibroblasts treated with H_2O_2 was shown to reduce the production of SASP components such as ICAM-1 and MMP-9 and the generation of ROS and to partially rescue senescence (Lee et al., 2010). In a model of premature senescence triggered by methoxypsoralen and ultraviolet-A irradiation (PUVA) in human dermal fibroblasts, PPARγ ligand-induced activation partially rescued the senescence phenotype, by controlling mitochondrial damage and ROS production (Briganti et al., 2014). Altogether, the observations reported so far on the action of PPARγ in senescence are quite controversial and therefore the effect of this receptor in this context is still a matter of debate which needs further investigation.

As mentioned earlier, there are three PPAR receptors (PPARα, PPARβ/δ and PPARγ). Although PPARα was not formally linked to senescence in the literature, its activation was reported to inhibit vascular smooth muscle cell proliferation *in vivo* in a p16-dependent manner. PPARα activation induces CDKN2A expression through both PPARα binding to PPAR-response elements in the CDKN2A promoter and PPARα interaction with the transcription factor SP1 at SP1-binding sites (Gizard et al., 2005). PPARβ/δ was shown to promote senescence induced by the RAS oncogene in keratinocytes by promoting phosphorylated extracellular signal-regulated kinase (p-ERK) and repressing phosphorylated protein kinase B (p-AKT) signaling (Zhu et al., 2014a) and by inhibiting ER stress (Zhu et al., 2014b).

Regarding TRα, our data (Fig. 2B–D) are the first to report its involvement in cellular senescence. Thyroid hormone T3 was shown to stimulate the expression of TFAM and genes of the mitochondrial respiratory chain such as UQCRFS1 by activating TRβ and inducing the recruitment of TRβ and NRF1 (nuclear respiratory factor 1) on the promoter of these genes. Increased mitochondrial respiration triggers the generation of ROS, which cause oxidative stress, oxidative DNA damage and cellular senescence in an ATM-dependent manner (Zambrano et al., 2014).

VDR knockdown decreases BRCA1 mRNA level (through transcriptional regulation) and 53BP1 protein level (through cathepsin L-mediated degradation), impairs their recruitment to DNA damage sites and thereby causes a deficiency in DNA repair leading to accumulation of DNA damage and cellular senescence (Graziano et al., 2016). This induction of senescence is accompanied by a high CDKN1A mRNA level, which can be due to DNA damage accumulation and subsequent p53 activation in this context. However, in a differential cDNA library screen for vitamin D3 responsive genes, CDKN1A was identified as a gene, the expression of which can be transcriptionally induced by

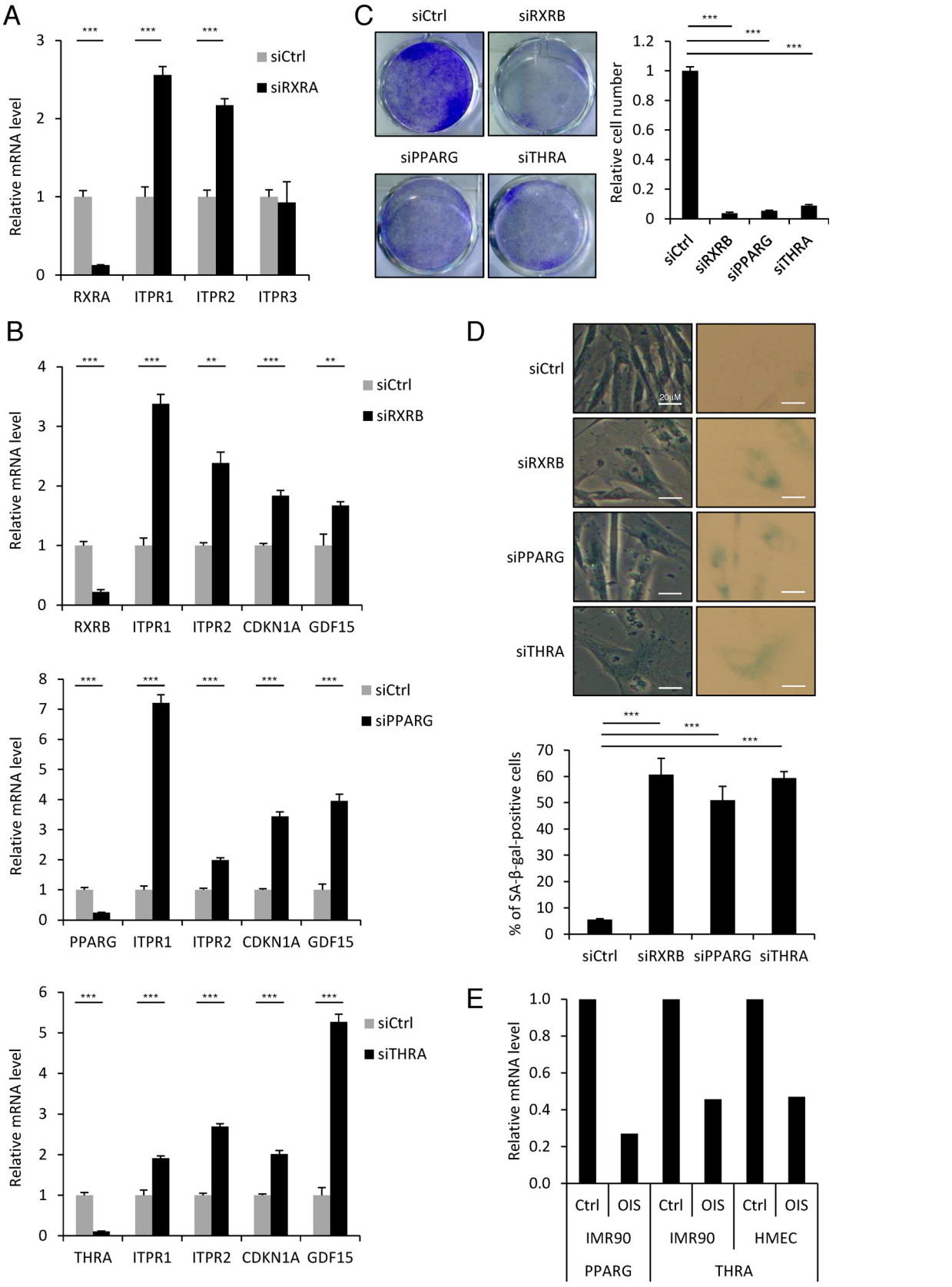

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Fig. 2. RXR receptors and some dimerizing partners regulate ITPR expression and cellular senescence. (a) Knockdown of the nuclear receptor RXRA induces the expression of ITPR1 and ITPR2 but not ITPR3. MRC5 primary human fibroblasts were transfected with a control siRNA pool (siCtrl) or a siRNA pool targeting RXRA (siRXRA). Four days later, the mRNA level of RXRA, ITPR1, ITPR2, and ITPR3 was checked by RT-qPCR. (b–d). Knockdown of the nuclear receptors RXRB, PPARG and THRA induces ITPR expression and cellular senescence. MRC5 were transfected with siCtrl pool or siRNA pools targeting RXRB, PPARG or THRA as indicated. (b) Four days later, knockdown efficiency and the mRNA level of ITPR1, ITPR2, CDKN1A and GDF15 were measured by RT-qPCR. (c). Six days after transfection, cells were stained with crystal violet (left) and counted (right). (d) On the same day, a SA-β-galactosidase assay was performed. Representative pictures (upper panel) and the percentage of SA-β-galactosidase positive cells counted in each condition (lower panel) are shown. The experiments shown are representative of at least three biological replicates. Errors bars show standard deviation. Statistical analysis was performed with Student's *t*-test (** for $P < 0.01$ and *** for $P < 0.001$). (e) Expression of the nuclear receptors PPARG and THRA is downregulated upon oncogene-induced senescence (OIS). Microarray data showing a decreased mRNA level of PPARG in primary human fibroblasts IMR90 induced into senescence by the RAS oncogene compared to control (Ctrl) (Takebayashi et al., 2015) and of THRA in primary human fibroblasts IMR90 induced into senescence by the RAS oncogene (Acosta et al., 2013) or in human mammary epithelial cells HMEC induced into senescence by the MEK oncogene (Warnier et al., 2018) compared to their respective control (Ctrl). Cell culture, siRNA transfection, RNA extraction, reverse transcription, real-time quantitative PCR, crystal violet staining and SA-β-galactosidase staining were performed as described in (Ma et al., 2018). Sequences of siRNAs are: GGGCAAUCAUUCUGUUUAA, GCAAACGGCUAUGUGCAAU, CGAAGAGGAUCCCACACUU and CCGCAAAGACCUUACAUAC for siRXRB pool, CAAAUC ACCAUUCGUUAUC, GACAUGAAUUCCUUAAUGA, GAUAUCAAGCCCUUCACUA and GACAGCGACUUGGCAAUAU for siPPARG pool, CGGCCAAUGUUCCCUGAAA, GAACUGGGCAAGUCACUCU, GUAUAUCCCUAGUUACCUG and GAACCUCCAUCCCACCUAU for siTHRA pool. Sequences of other siRNAs are listed in (Ma et al., 2018). Primer sequences for real-time qPCR are: 5′-tacccagcggctgctaac-3′ and 5′-tgcaaatcctgctcctctgt-3′ for ITPR1, 5′-ctgctgtagccagtgcagac-3′ and 5′-ggagcaa-gatcgtccatca-3′ for ITPR3, 5′-aggtgggtgtttgggaaag-3′ and 5′-ccccaagttagacaggaagagata-3′ for RXRB, 5′-gacaggaaagacaacagacaaatc-3′ and 5′-ggggtgatgtgtttgaacttg-3′ for PPARG, 5′-ggctgtgctgctaatgtcaa-3′ and 5′-tgactcttctcgatcttgtcca-3′ for THRA. Other qPCR primer sequences are listed in (Ma et al., 2018).

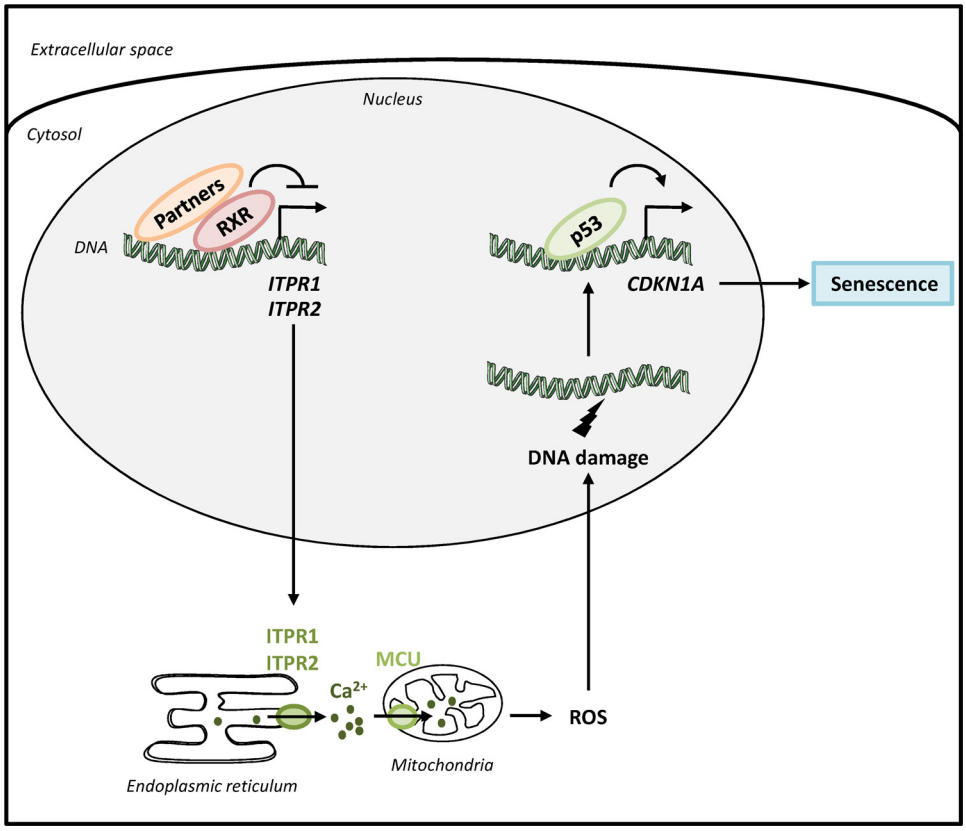

Fig. 3. Model for senescence regulation by RXRs and their partners through calcium signaling. RXRs directly repress the expression of ITPR1 and ITPR2 and thereby control calcium release from the endoplasmic reticulum, calcium accumulation in the mitochondria through MCU, generation of reactive oxygen species, DNA damage, activation of p53, induction of its target gene CDKN1A encoding the cyclin-dependent kinase inhibitor p21 and finally implementation of cellular senescence. Partners of RXRs such as PPARγ and TRα could take part in this regulation.

vitamin D3 in a VDR-dependent and p53-independent manner (Liu et al., 1996). Three promoter regions bound by the VDR-RXR heterodimer, containing vitamin D response elements and mediating this induction, were further identified in the CDKN1A promoter (Saramaki et al., 2006). The VDR nuclear receptor is thus able to regulate the expression of CDKN1A both directly and indirectly. In addition to CDKN1A, which encodes p21, expression of CDKN1B encoding the related CDK inhibitor p27 was upregulated in response to vitamin D3, as well as the expression of CDKN2A, CDKN2B and CDKN2C (Liu et al., 1996). However, the effect of vitamin D3 and action of VDR in senescence require further investigation as vitamin D was reported to delay senescence in cells derived from Hutchinson-Gilford Progeria Syndrome (HGPS) patients. Vitamin D3 was shown to reduce progerin production by regulating LMNA gene expression and to promote the stabilization of BRCA1 and 53BP1, reducing DNA repair defects in these cells (Kreienkamp et al., 2016).

Other type II nuclear receptors known to heterodimerize with RXR receptors have also been implicated in cellular senescence. Activation of liver X receptors (LXRs) by ligands was reported to inhibit cellular senescence induced by hyperglycemia in endothelial cells through reduction of ROS production and induction of endothelial NO synthase (eNOS) expression (Hayashi et al., 2014). RAR ligands, both agonist and antagonist, were shown to trigger senescence and paracrine growth inhibition in MCF-7 breast cancer cells (Chen et al., 2006). Additional works are needed to understand the molecular mechanisms underlying this observation.

Altogether, RXRs and their partners are able to control cellular senescence through multiple mechanisms, including regulation of calcium signaling, ROS, DNA damage, CDKN1A and CDKN2A expression (Fig. 4). Of note, in light of our recent findings, regulation of calcium

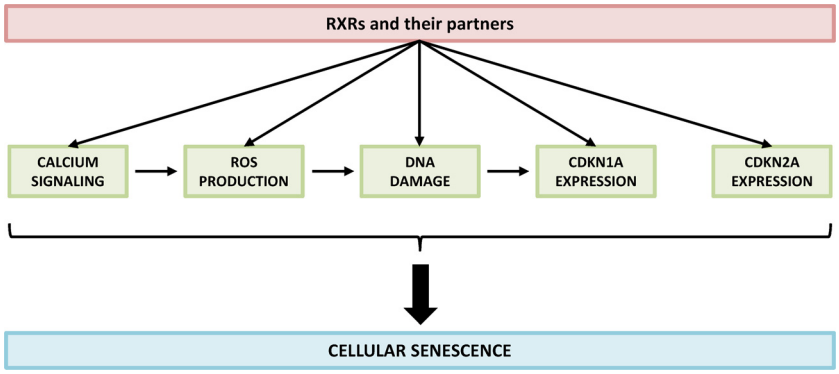

Fig. 4. Known mechanisms involved in the control of cellular senescence by RXRs and their partners. RXRs and their partners regulate the expression of CDK inhibitors CDKN1A and CDKN2A through various mechanisms. Other mechanisms, yet to be identified, may contribute to senescence regulation by nuclear receptors.

signaling could be involved in the regulation of ROS, DNA damage and CDKN1A reported for partners of RXRs. Although our understanding of the involvement of these nuclear receptors in senescence has significantly improved over the last decade, the difficulty to master ligand concentration hampers the interpretation of experimental observations. This could be a reason for data discrepancy, together with differences in cell types and cell lines used. Further studies are required to clarify the action of these receptors as well as their role in different contexts of senescence, *in vitro* and *in vivo*.

5. Regulation of RXRs and their partners in senescence

Whether RXRs and their partners are regulated upon cellular senescence, and the mechanisms underlying such regulation, are barely known. We did not find any variation in RXRA and RXRB expression following senescence in different contexts. However, the expression of several nuclear receptors which heterodimerize with RXRs changes upon senescence. We observed that the mRNA level of THRA and PPARG decreased upon oncogene-induced senescence (Fig. 2E). PPARG expression was also downregulated upon premature senescence induced by PUVA (Briganti et al., 2014) or H2O2 (Lee et al., 2010) and upon replicative senescence in late passage fibroblasts (Lee et al., 2010). VDR expression also decreased upon oncogene-induced senescence (Graziano et al., 2016) and in cells derived from HGPS patients (Kreienkamp et al., 2016). Protein level and activity of nuclear receptors can also be regulated. PPARγ transactivation activity was found to be modulated in senescent cells (Briganti et al., 2014). A decrease in its phosphorylation was reported in this context (Gan et al., 2008). Increased acetylation of PPARγ was also observed with increasing passage number, the acetylation status of this nuclear receptor being regulated by the acetylase p300 and deacetylase SIRT1 (Han et al., 2010). Moreover, transcriptional activity of nuclear receptors is modulated by their interaction with transcriptional co-regulators. Some of them, including SMRT, have been shown to control cellular senescence (Reilly et al., 2010). Senescence-associated variations in the expression and protein level of nuclear receptors as well as in their activity (regulated by post-translational modifications and interacting partners) should be more thoroughly investigated.

6. Role of RXRs and their partners in senescence-associated physiopathological contexts

Several of these nuclear receptors are implicated in physiopathological contexts known to be regulated by cellular senescence such as cancer, aging and aging-associated diseases. For example PPAR receptors, which regulate senescence as described above, control aging *in vivo*. Mice lacking PPARα display decreased longevity and an enhancement of age-dependent lesions in the liver, kidney and heart as well as hepatocellular adenomas and carcinomas (Howroyd et al., 2004). PPARβ/δ regulates age-related macular degeneration (Choudhary et al., 2016). Mice lacking PPARγ isoforms 1 and 2 in the white adipose tissue or of PPARγ isoform 2 in all tissues also have a reduced lifespan (Argmann et al., 2009). Ablation of PPARγ exacerbates age-associated obesity and metabolic dysfunction (Xu et al., 2018). Thyroid hormones, which can trigger senescence as mentioned earlier, accelerate aging *in vivo* (Zambrano et al., 2014). Inhibition of endothelial cell senescence was proposed to underlie the protecting effect of LXRs against atherosclerosis (Hayashi et al., 2014). Further studies will need to determine if and to which extent the effect of nuclear receptors observed *in vivo* depends on their impact on cellular senescence.

7. Concluding remarks

Retinoid X receptors and their partners have now emerged as potential regulators of cellular senescence. Beyond the mechanisms described here, it is very likely that additional modes of action, yet to be identified, underlie their effect in this process. For instance, we could hypothesize that several nuclear receptors could control senescence by directly modulating the expression of SASP components. As mentioned earlier, regulation of these receptors upon cellular senescence should be studied. Moreover, and importantly, the contribution of the regulation of senescence to their role *in vivo* should be investigated.

Beyond RXRs and their partners, several other nuclear receptors have been characterized as key regulators of cellular senescence in the last ten years. For example, the chicken ovalbumin upstream promoter-transcription factor β COUP-TFβ (NR2F2) (Zhu et al., 2016), the tailless homolog orphan receptor TLX (NR2E1) (O'Loghlen et al., 2015; Wu et al., 2015) and the testicular orphan receptor 4 TR4 (NR2C2) (Lee et al., 2011) have been shown to critically regulate this process. For a better understanding of the role of nuclear receptors in this context, the ability of all the members of the nuclear receptor superfamily to control senescence should be assessed, for example by siRNA screening.

As nuclear receptors bind to small molecules, they represent promising pharmacological targets for agonists and antagonists. A number of molecules targeting nuclear receptors are already in use in clinics and many new ones are under investigation. For instance, pharmacological activation of REV-ERB nuclear receptors (NR1D1 and NR1D2) was recently shown to be lethal for cancer cells and cells in oncogene-induced senescence without affecting normal cells, to impair glioblastoma growth *in vivo* and extend survival (Sulli et al., 2018).

As cellular senescence plays a crucial role in many physiopathological contexts, it is of high importance to improve our knowledge of the nuclear receptors regulating this process and our understanding of the mechanisms underlying their action. Nevertheless, as they can both activate and inhibit transcription of the same target genes pending on ligand bioavailability, genetic and pharmacological manipulation of RXRs and of their ligands can result in opposite outcomes. Therefore, determining their functions and establish conclusions *in vivo* is a

challenging issue.

Declaration of Competing Interest

None declared.

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