show that multiple miRNAs such as miR-17-5p, miR-20a/b, miR-93, miR-106a/b, miR-146a-b, miR-372 and miR-373 bypass Ras-induced senescence by directly targeting p21 [27]. Therefore, one miRNA can regulate several genes to cooperate in senescence, whereas many miRNAs can target one gene to control senescence (Fig. 1).

Traditionally, cellular senescence has been thought to arise as a consequence of altered gene expression including senescence genes, senescence suppressor genes, oncogenes, tumor suppressor genes, stemness genes, epigenetic genes and others (Table. 1). Cellular senescence can be triggered by telomere shortening due to the end replication problems in human somatic cells. Telomerase, a ribonucleoprotein enzyme for telomere elongation, is inactivated in normal somatic cells, but most cancer cells and stem cells have telomerase activity due to the activation of human telomerase reverse transcriptase (hTERT) gene. Although little is known about hTERT regulation by miRNAs, it is reported that miR-138 potentially targets the hTERT gene, involved in the development of thyroid carcinoma [49]. miR-128a was identified to directly target Bmi-1 oncogene, increasing p16 expression to promote cellular senescence [50]. The p53-independent up-regulation of miR-34a provoked senescence through repression of v-myc myelocytomatosis viral oncogene homolog myc [51]. miR-372 and miR-373 prevent Ras-induced senescence through the down-regulation of tumor suppressor LATS2 [52]. miR-21 modulates apoptosis by regulating Ras [53] and phosphatase and tensin homolog (PTEN)-dependent activation of PI 3-kinase signaling [54]. The miR-302-367 cluster acts after Oct4, Sox2 and Nanog to maintain stemness of human embryonic stem cells [55]. Let-7b is involved in decline of neuronal stem cell self-renewal during aging by reducing HMGA2 levels in old but not in young mice [56]. In addition, miR-146a can inhibit cellular senescence by targeting IL-6/8 [57]. The proto-oncogene LRF, leukemia/lymphoma related factor, is post-transcriptionally regulated by miR-20a, inducing senescence in mouse embryonic fibroblasts through preventing p19ARF transcription [16]. miR-29 and miR-30 can mediate repression of B-Myb expression during Rb activation, resulting in cellular senescence [58]. Some receptors such as ER [59] and ERBB2/3 [60] are regulated by miR-22 and miR-125, respectively. Above all, these miRNAs modulate cellular senescence through targeting various genes on p53/pRB pathway and/or other signaling pathways.

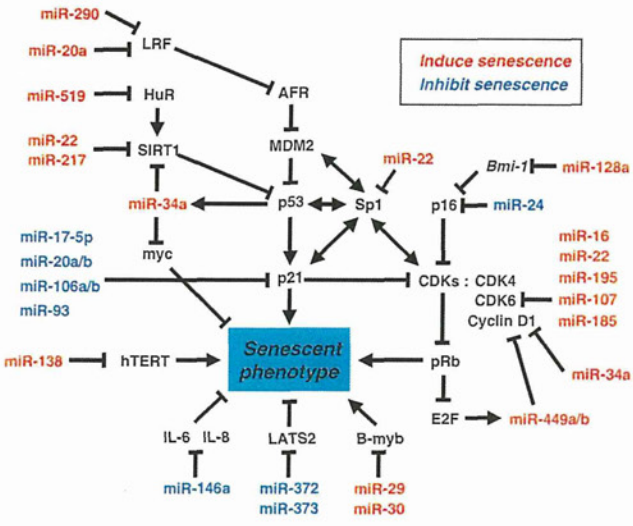

Fig. 1. miRNAs are involved in cellular senescence through p53/pRb pathway and other pathways. Cellular senescence is modulated by a variety of miRNAs as shown. These regulatory miRNAs induce senescence phenotypes (indicated in red) or delay the onset of senescence (indicated in blue).

Table 1
Genes associated with cellular senescence.

Genes	Functions	Examples	miRNAs	References
Senescence genes	Cell cycle regulators	p16	miR-24	[46]
		p21	miR-106a/b	[27]
Senescence suppressor genes	Regulators of telomere length	hTERT	miR-138	[49]
Oncogenes	Signal transduction molecules	Ras	miR-21	[53]
		Bmi-1	miR-128a	[50]
		myc	miR-34a	[51]
Tumor suppressor genes	Signal transduction molecules	p53	miR-34a	[44]
		LATS2	miR-372, 373	[52]
		PTEN	miR-21	[54]
Stemness genes	Self-renewal regulators	SOX2	miR302-367	[55]
		OCT3/4		
Epigenetic genes	Chromatin factors	HMGA2	Let-7b	[56]
	Histone modification	SIRT1	miR-22	[17]
Others	Inflammatory factors	IL-6, 8	miR146a	[57]
	Transcription factors	LRF	miR-20a	[16]
		Sp1	miR-22	[17]
		B-Myb	miR-29, 30	[58]
	Receptors	ER	miR-22	[59]
		ERBB2/3	miR-125a-5p	[60]

4. Involvement of secretory factors in cellular senescence

Senescent cells secrete not only exosomes but also other inflammatory cytokines and chemokines [61]. Recently, it has been reported that senescent cells assume a novel phenotype, the so-called senescence-associated secretory phenotype (SASP), which developed in normal fibroblasts, epithelial cells, and epithelial tumor cells after genotoxic stress in culture, and in epithelial tumor cells in vivo after treatment of prostate cancer patients with DNA damaging chemotherapy [62]. SASP is characterized by the secretion of myriad factors associated with inflammation and malignancy factors as well as the release of exosomes [21,43,62].

The secreted factors including matrix components, extracellular proteases, cytokines, and chemokines set the stage for crosstalk between senescent cells and their environment [63]. Of note, the effect of secreted factors is complex: they seem to reinforce the senescent phenotype in nearby senescent cells but at the same time can increase the proliferation of nearby neoplastic cells. For example, interleukin (IL)-6 contributes to the induction of senescence of nonmalignant cells in an autocrine manner [64], whereas its tumor promoting function by paracrine signaling has been proposed to directly or indirectly target the angiogenic compartment [65]. In cultured premalignant epithelial cells, SASPs induced an epithelial–mesenchyme transition and invasiveness, hallmarks of malignancy, by a paracrine mechanism that depended largely on the IL-6 and IL-8. Senescent cells contribute to induction of senescence in cells in its immediate vicinity mediated by secreted factors, which could constitute a danger signal that sensitizes normal neighboring cells to enter senescence, and block growth factors signaling directly by inhibition at the receptor level, thereby potentially contributing to the induction of senescence [66]. Tumor cells can misuse signaling of secreted factors, inducing senescence in fibroblasts. These senescent fibroblasts could in turn secrete factors that contribute to tumor growth in the later stage of tumorigenesis [67]. In addition, aging can lead to an increase in the number of senescent cells, thereby potentially stimulating expansion of premalignant cells [40]. Therefore, it is thought that cellular senescence suppresses tumorigenesis early in life, whereas it may promote cancer in aged organisms.

In addition to secretion of cytokines, senescent cells produce high levels of exosomes that are able to modulate the microenvironment during senescence. Exosomes are secreted by most cell types including dendritic cells, B cells, T cells, epithelial cells, and tumor cells, and interact with surrounding cells upon secreted. Exosomes can introduce

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regulatory secreted factors into the interiors of the cells or receptors into the external membranes of the cells, providing intercellular communication both within and between cells [6]. Exosomes participate in the surface-membrane traffic and the horizontal transfer of protein and RNAs as well miRNAs among neighboring cells, which are necessary for the rapid phenotype adjustments in a variety of conditions. [68]. It is reported that senescence is associated with the release of exosomes, demonstrating a significantly increased release of exosome-like microvesicles during senescence in normal human fibroblasts. Importantly, senescence-associated exosomes were also observed to release from human senescent prostate cancer cells, suggesting senescence-associated exosomes can transfer cargos with both immunoregulatory potential and genetic information, between cells during cellular senescence [19]. EGFR is repressed at the chromatin level during senescence and is shown to be potentially regulated during cellular senescence [69]. Exosome-like microvesicles released by EGFR-bearing tumor cells are incorporated by neighboring endothelial cells, resulting in activation of the MAPK and Akt pathways and the autocrine production of VEGF. Their transfer between EGFR-bearing glioma cells can accelerate the growth of tumor cells that take up the vesicles. Thus, the effects of exosomal release in senescence could be wide ranging and influence the microenvironment [70].

The secretory activity and the release of exosomes in senescent cells are regulated by p53 tumor suppressor (Fig. 2) [71]. In cells undergoing senescence, p53 acts as a transcription factor and up-regulates a variety of secreted factors such as insulin-like growth factor-binding protein 3 (IGFBP-3). IGFBP3, secreted by some senescent cells, is known to be up-regulated in senescent fibroblasts and endothelial cells, leading to senescence [71,72]. In addition, it is reported that p53 up-regulates tumor suppressor-activated pathway-6 transcript (TSAP6), which is an intracellular factor that stimulates exosome release into the extracellular medium [71,73]. Chmp4 that recruits Alix protein to the endosome to function in MVB vesicle formation is also induced by p53 activation [74]. The p53 protein can also activate caveolin-1, the main component of the caveolae plasma membranes, and facilitate the endocytosis, accompanied with endosomal clearance of membrane receptors from cell surface [74,75] . In addition, overexpression of caveolin-1 induced premature senescence in human fibroblasts [76], whereas knockout of caveolin-1 by shRNA blocks the induction of bleomycin-induced senescence [77]. Maspin, a mammary serine protease inhibitor, is also up-regulated by p53 in response to stress, and plays a role in the exosome secretion [71]. At present, it is well known that both secreted factors and exosomes are secreted from senescent cells, and involved in a variety of biological function, but it is still unclear whether they coordinately influence each other for the biological effects of age-associated diseases.

5. Biological processes mediated by miRNAs and exosomes during senescence

Recent findings have revealed the complexities of the senescence phenotypes, demonstrating that senescent cells differ from proliferating cells in three way: (1) they arrest cell growth and cannot be stimulated to reenter the cell cycle by known physiological stimuli; (2) they become resistant to apoptotic cell death; (3) they acquire altered differentiated functions [21,78]. All of these changes might result from cooperative changes in multiple systems with the resultant changes to pathways regulating different cellular processes.

The ability of miRNAs to regulate a variety of target genes allows them to induce changes in multiple biological processes, implicating that miRNAs may provide a significant link between senescence and other biological processes. Using the systems biology tool Metacore from Genego, pathway analysis of senescence-associated miRNA targets demonstrates the common signaling pathways resulting in senescence [79]. Dhahbi et al. used DAVID and Gene Ontology to functionally annotate miRNA-regulated genes, revealing the biological processes targeted by miRNA expression changes during senescence [80]. Among them, the most highly represented and enriched term is regulation of cell proliferation, which is consistence with a pivotal role of miRNAs in cell proliferation regulation since inhibition of proliferation is the hallmark of senescence [21]. Senescence-associated miRNAs, miR-22 and miR-34a

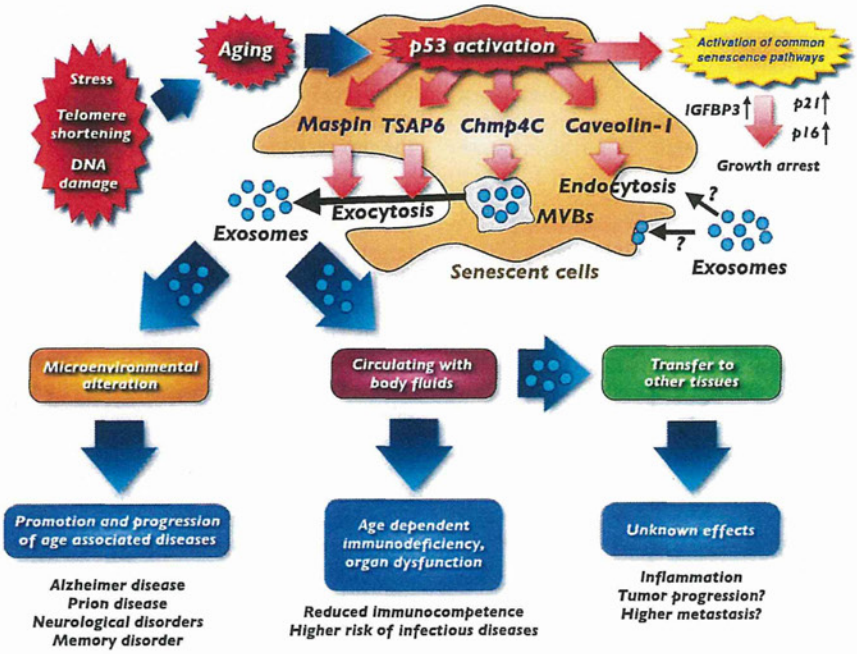

Fig. 2. The secretory activity and the release of exosomes are regulated by p53 tumor suppressor in senescent cells. Senescence-inducing factors such as oxidative stress, telomere shortening and DNA damage induce p53 activation followed by the increase in p21, p16 or IGFBP3 on the senescence pathways. The activation of p53 also induces up-regulation of Maspin, TSAP6, Chmp4C and Caveolin-1, which can enhance the endosome production, the exosome production and release from the plasma membrane. Higher production of exosomes which cargo miRNA and proteins from senescent cells may induce the alteration of microenvironment in age-associated diseases, cause immunodeficiency and organ dysfunctions in infectious diseases, and result in other unknown effects such as inflammation.

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inhibit cell proliferation by targeting common senescence pathways [17,18]. Other miRNAs also contribute to regulate cell proliferation through common signaling pathways of senescence (Fig. 1). Notably, miRNAs regulate actin cytoskeleton structure changes that contribute to the enlarged and flattened cell morphology characteristic of senescence phenotype. Our previous study demonstrates that miR-22 induces senescent-like morphology in SiHa cells, accompanied with cytoskeleton remodeling such as actin fiber formation [17]. miRNAs control key molecules in the multiple processes governing the cell cycle during senescence induction, which allows these miRNAs to affect the cell cycle regulatory process as whole rather than at just one checkpoint or stage [79]. Because the capability of cells to adhere to extracellular matrix is linked to cytoskeleton remodeling and cell shape, the changes in cell adhesion regulation may contribute to the morphological changes induced in senescence. Senescent cells are known to acquire resistance to apoptotic stimuli, whereas miRNAs may sway the cellular decision to commit to senescence instead of apoptosis. Alteration of protein metabolism and modification is responsible for the increase in cell volume and mass resulting in the enlarged and flattened morphology. So far, various studies support that miRNAs could facilitate the complex cellular changes required to establish the senescent phenotype, associated with inhibition of cell proliferation, cellular metabolic processes, negative regulation of apoptosis, stimulation of cell adhesion and cytoskeleton remodeling. Here, we summarize the effects of several miRNAs, either up or down in senescence, on biological processes changed during cellular senescence (Table. 2).

Exosomes are secreted into surrounding body fluids, targeting cells to transfer exosomal molecules including proteins, mRNAs, and miRNAs that can aid cell proliferation, immune response and other cellular process. For example, exosomes released by prematurely senescent prostate cancer cells were enriched in B7-H3 protein [19], which is a member of the B7 family of proteins that are capable of modulating CD4 T-cell responses and antitumoral immunity [81]. Secreted factors IL-6/8 from senescent fibroblasts in SASP are involved in the induction of a classic epithelial–mesenchyme transitions and invasiveness in human breast cancer cell lines [62]. Previous studies have found miRNAs circulating in the peripheral blood and have identified exosomes as mediators of cell–cell communication. It is reported that macrophage-secreted exosomes would deliver oncogenic miRNAs to breast cancer cells, contributing to the regulation of the invasiveness of breast cancer cells [82]. Therefore, exosome formation, cargo content, and delivery to surrounding cells are thought to be of immense biological interest in cellular processes. Little is known about the role of exosomes in vivo aging; however, these in vitro experimental data give the biological importance of exosomes in aging.

6. Potential of exosomes and miRNAs in aging and cancer

Aging is a well-recognized risk factor in the development of many diseases such as neurodegenerative disorders, cardiovascular disease, diabetes and cancer, which are the primary cause of death and disability in the elderly population. The process of aging impacts on a wide range of functions within the human body, displaying age-related changes occur in cellular, molecular and physiological functionality of tissues and organs. Nowadays, it is a well-known fact that some age-related molecular changes (e.g. DNA methylation, telomere shortening) can be detected in several types of colorectal cancers [83]. The understanding connection of aging process to age-related diseases is of great clinical importance regarding prevention and modern therapeutic strategies.

Exosomes are believed to play a role in various physiological and pathological conditions. Senescence-associated exosomes could transfer many regulatory factors including proteins and miRNAs, which contribute to the aging process in an autocrine, paracrine and even endocrine fashion. Although the relationship between miRNA and aging is not fully understood, the accumulating evidence suggests that exosomes act as significant communicator in human body. Exosomes have been implicated in the spread of neurodegenerative diseases including age-related macular degeneration (AMD) and Alzheimer's disease (AD). Age-related macular degeneration (AMD) is a major cause of loss of central vision in the elderly. Wang et al. demonstrated that increased autophagy and the release of intracellular proteins via exosomes by the aged retinal pigment epithelium may contribute to the formation of drusen that is a risk factor for developing AMD [84]. Alzheimer's disease (AD) is an irreversible age-related degenerative disorder, characterized by amyloid plaques, neurofibrillary tangles, inflammation, and neuronal damage in the brain. Accumulation of β-amyloid peptides (Aβ) in the senile plaques is a hallmark of the progression of disease. Interestingly, Aβ peptides formed by the β cleavage of amyloid precursor protein (APP) by two unknown proteases, β-secretase and γ-secretase, are secreted from the cells in association with exosomes [85]. Similar observation was found in prion diseases, which are fatal and infectious neurodegenerative disorders characterized by the accumulation of an abnormally folded form

Table 2
Analysis of biological processes changed during cellular senescence.

	Up-regulated miRNAs			Down-regulated miRNAs		
Biological processes	Let-7	miR-22	miR-34a	miR-17	miR-21	miR-155
Cell proliferation	Inhibited	Inhibited	Inhibited	Promoted	Promoted	Promoted
Apoptosis	Inhibited	Inhibited	Induced	Inhibited	Induced	Inhibited
Cell cycle arrest	G1/S arrest G2/M arrest	G1/S arrest	G1/S arrest G2/M arrest	Inhibitor of miR-17 induces G0/G1 arrest	Antisense targeting miR-17 induces G1 arrest	Antisense targeting miR-155 induces G0/G1 arrest
Cell differentiation	Involved	Involved	Involved	Involved	Involved	Involved
Metabolic processes	Involved	Inhibited	Inhibited	Involved	Involved	ND
Protein metabolism and modification	Involved	Involved	Involved	Involved	Involved	Involved
Cell adhesion	ND	Involved	decreased	decreased	Involved	decreased
Cytoskeleton remodeling	ND	Increased	ND	ND	Involved	ND
Cell migration and cell motility	Inhibited	Inhibited	Inhibited	decreased	Involved	Inhibited

Note: all the data are summarized from published articles. ND means not determined.

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of glycosylphosphatidylinositol-linked prion protein (PrP), scrapie PrP [86–88]. These results suggest that exosomes might affect the microenvironment of these age-related diseases.

It is found that miRNAs can be stably detected in circulating plasma and serum, since miRNAs are packaged by exosomes to be protected from RNA degradation [13]. Ago2 and HDL that are associated with miRNAs are also stably found in serum, but their biological significance is still being discussed [89–91]. While miRNA profiling between cells and exosomes seems to be different, similar miRNA profiling was observed in exosomes containing miRNAs and Ago2 binding miRNAs. Therefore, the functional regulation of exosome secretion to deliver specific miRNAs may exist during aging and in human diseases. Recent studies have indicated that circulating exosomal miRNAs can serve as surrogates for the tumor cell-associated counterparts, extending their diagnostic potential to asymptomatic individuals [92]. Considering the high relevance between exosomes and miRNAs in many aspects, we suppose that circulating miRNAs will have a potential role in aging and age-related diseases.

Loss of immune function is typical symptom in human aging. Among human body fluids, one of major body fluid is blood, and a potential major in vivo source of exosomes is the B cell. Little is known about the function and the possible targets of B cell-derived exosomes. Exosomes derived from B cells in both human and mice elicit immune response by inducing antigen-specific MHC class II-restricted T-cell responses [93]. The frequency and number of bone marrow common lymphocyte progenitors (CLPs) are considerably reduced by 7 months of age. Furthermore, while about 90% of CLPs numbers is reduced in 20 month old mice, age-dependent reduction of the frequency and number of pro-B cell is observed. Due to recombination of impaired V-DJ heavy chain gene, the transition of pro-B cell into pre-B cells also decreases with aging. It is possible that these alterations may reflect on profiling and secretion of exosomes from these cells.

Studies on tumor derived exosomes reveal that exosomes may help establish an oncogenic niche systemically via delivery of protein, mRNA, and miRNAs that can aid tumor growth and metastasis. The presence of HLA-G in exosomes secreted by melanoma cells might imply a role in creating a tumor tolerance associated response [94]. Utilizing VEGF and cytokines cargo, exosomes enhance recruitment of hematopoietic and endothelial precursor cells to enhance neo-angiogenesis in the tumor [95]. Additionally, exosomes are known to trigger apoptotic reduction in anti-tumor immune cells through FasL and TNF-pathways and influence planar cell polarity and the extracellular matrix to allow tumor cell mobilization [96].

In summary, exosomes allow for reciprocal communication between different tissues, as shown in Fig. 3. Exosome production and secretion are altered during in vivo aging and in cancer, which has biological significance in metabolism, glycolysis, renal function, immunocompetence, tumor progression and brain memory function. Secretory factors are circulating in the tissues through the blood, so they may impact on the phenotypes by coordinate alteration of miRNAs, exosome proteins, lipids and cytokines. Furthermore, it is possible that individual difference in the production of secretory factors during aging may reflect the cancer risk as well as the cure rate. To date, limited information is available for understanding the mechanism and function of age-associated exosomes. We suppose that alteration of circulating miRNA, RNA, lipids and proteins via exosomes may coordinately influence many biological processes in human aging and cancer.

There is an increasing interest in using miRNAs as biomarker in the diagnosis of diseases and also as a promising biomedicine in the therapy of human diseases. Recent study shows that the sensitivity of miRNA amplification from human biologic fluids such as serum and saliva can be improved by exosome isolation [97], indicating exosomal miRNAs might become a potential starting point for earlier

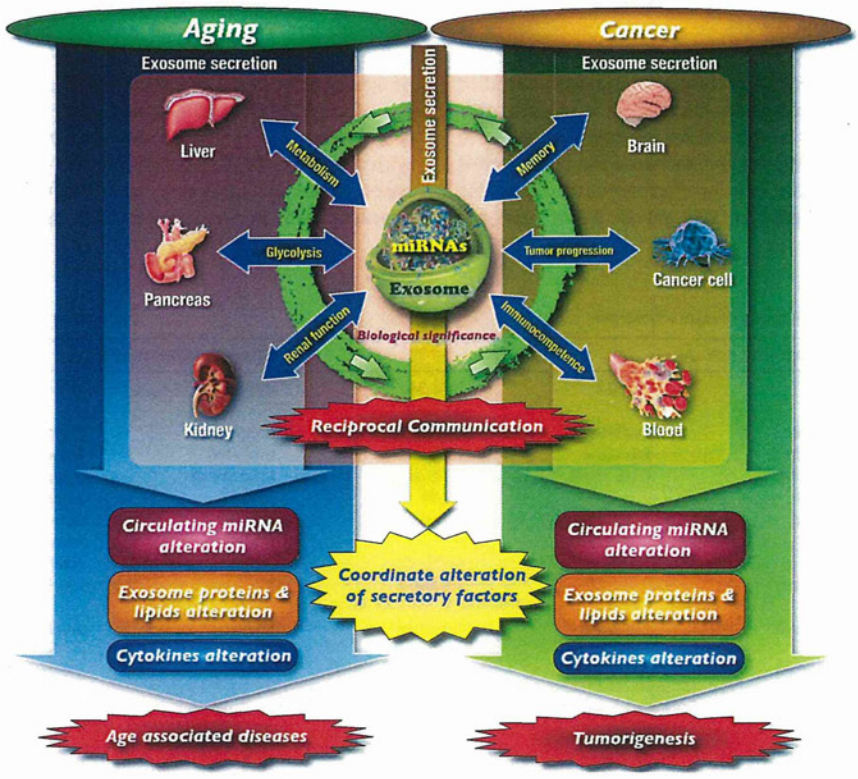

Fig. 3. Exosomes that allow for reciprocal communication between different tissues are critical in coordinating regulation of cellular function through miRNAs and proteins transfer. Epigenomic alterations during aging or in cancer may increase exosome secretion, accompanied with alteration in expression of circulating miRNAs, exosome proteins and cytokines, which are involved in cell-to-cell communication via exosomes. Therefore, exosomes play an important role in age-associated diseases and tumorigenesis.

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biomarker studies to reduce the probability of false negative results in clinic diagnosis of human diseases.

Nowadays, therapeutic potential of RNA drug to the clinic has been realized. With the development of nonimmunogenic delivery technologies, it is reported that exosomes can be used as nanocarriers to mediate siRNA delivery to the brain in mice, demonstrating efficient knockdown of BACE1, a therapeutic target in Alzheimer's disease [98]. In conclusion, exosomes provide a novel biomarker discovery and therapeutic platform for delivery of a variety of therapeutic modalities with great promise.

Acknowledgements

This work is supported by Project for Development of Innovative Research on Cancer Therapeutics and Grant-in-Aid for Scientific Research (KAKENHI), Ministry of Education, Culture, Sports, Science and Technology (H. Tarara) in Hiroshima University and "the Fundamental Research Funds for the Central Universities 2012TD027" in Dalian Maritime University.

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Please cite this article as: D. Xu, H. Tahara, The role of exosomes and microRNAs in senescence and aging, Adv. Drug Deliv. Rev. (2012), doi:10.1016/j.addr.2012.07.010

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Please cite this article as: D. Xu, H. Tahara, The role of exosomes and microRNAs in senescence and aging, Adv. Drug Deliv. Rev. (2012), doi:10.1016/j.addr.2012.07.010

nature genetics

Mutations in *UVSSA* cause UV-sensitive syndrome and destabilize ERCC6 in transcription-coupled DNA repair

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UV-sensitive syndrome (UV^{S}S) is an autosomal recessive disorder characterized by photosensitivity and deficiency in transcription-coupled repair (TCR), a subpathway of nucleotide-excision repair that rapidly removes transcription-blocking DNA damage[1]. Cockayne syndrome is a related disorder with defective TCR and consists of two complementation groups, Cockayne syndrome (CS)-A and CS-B, which are caused by mutations in *ERCC8* (*CSA*) and *ERCC6* (*CSB*), respectively[2]. UV^{S}S comprises three groups, UV^{S}S/CS-A, UV^{S}S/CS-B and UV^{S}S-A, caused by mutations in *ERCC8*, *ERCC6* and an unidentified gene, respectively[3–6]. Here, we report the cloning of the gene mutated in UV^{S}S-A by microcell-mediated chromosome transfer. The predicted human gene *UVSSA* (formerly known as *KIAA1530*)[7] corrects defective TCR in UV^{S}S-A cells. We identify three nonsense and frameshift *UVSSA* mutations in individuals with UV^{S}S-A, indicating that *UVSSA* is the causative gene for this syndrome. The UVSSA protein forms a complex with USP7 (ref. 8), stabilizes ERCC6 and restores the hypophosphorylated form of RNA polymerase II after UV irradiation.

To clone the gene responsible for UV^{S}S-A, mouse chromosomes were randomly transferred to Kps3 cells (UV^{S}S-A) by microcell-mediated chromosome transfers (MMCTs)[9] using mouse A9 cells as the donor. Kps3 cells fused with A9 microcells were UV irradiated periodically over 6 weeks. We obtained four independent UV-resistant cellular clones (15A-7, KAB1-14, KAGA2-6 and KAGB2-4) that exhibited normal levels of UV sensitivity and recovery of RNA synthesis after UV irradiation (UV-RRS) (**Supplementary Fig. 1**). To identify the mouse genomic DNA that was integrated in each clone, a comparative genomic hybridization (CGH) array analysis was performed. An intact mouse chromosome 5 was found in KAB1-14, whereas chromosome 5 with segments of chromosomes 12 and 17 was found in KAGA2-6. Only a few fragments of chromosome 5 were found in the 15A-7 and KAGB2-4 clones (**Fig. 1a**). These results indicated that the causative gene was located within the common 600-kb region of chromosome 5 that was integrated in the 15A-7 and KAGB2-4 clones (**Fig. 1b**). There are 11 known genes in that region (**Fig. 1c**). Six BAC clones[10] encompassing the 600-kb region were introduced into Kps3 cells (**Fig. 1c**). Kps3 clones transfected with BAC 476L03 acquired normal levels of UV resistance, whereas transfection with BAC 154C21 failed to complement UV-sensitive cells (**Fig. 1d**). These results indicate that *4933407H18Rik* is a complementing gene for UV^{S}S-A. The *4933407H18Rik* gene is the mouse homolog of a predicted human gene, *KIAA1530*, which has subsequently been renamed *UVSSA* (encoding UV-stimulated scaffold protein A) in light of this finding, with support from the Human Gene Nomenclature Committee (HGNC).

Sequencing of *UVSSA* cDNA in UV^{S}S-A cells revealed that Kps3 (Japanese)[4] and XP24KO (Japanese)[11] cells had a homozygous c.367A>T transversion, resulting in premature termination at amino acid 123 (p.Lys123*). TA-24 (Iranian)[3] cells had a homozygous deletion, c.87delG, that caused a frameshift at position 29, leading to premature termination at amino acid 39 (p.Ile31Phefs*9) (**Fig. 2a,b**). In addition, the expression of cDNA encoding Flag-HA-UVSSA successfully conferred normal levels of UV-RRS and UV resistance to Kps3 cells (**Fig. 2c,d**). Taken together, these results indicate that *UVSSA* is the causative gene for UV^{S}S-A.

The *UVSSA* gene is located on human chromosome 4 at p16.3 and is 40.7 kb long. The transcript of the gene is 4,336 nt long and is composed of 14 exons, encoding a protein that consists of 709 amino acids with a deduced molecular weight of 81 kDa[7,12]. Analysis of the amino-acid sequence of UVSSA revealed that there is a Vps27, Hrs and STAM (VHS) domain and a structurally similar epsin NH_2-terminal homology (ENTH) domain in the N-terminal region and a well-conserved domain of unknown function (DUF) 2043 domain in the C-terminal region (**Fig. 2a**). It was reported that the VHS/ENTH domains of SCAF8 and NRD1 interact with the C-terminal domain of RNA polymerase II (RNA Pol II)[13,14], suggesting that UVSSA also interacts with RNA Pol II. Homologs of UVSSA have been identified in *Arabidopsis thaliana*, rice and *Caenorhabditis elegans*, but there are no reports of their function.

To elucidate the function of UVSSA in TCR, we first examined its interaction with other TCR factors (**Fig. 3**). The UVSSA protein complex was affinity purified from the soluble or chromatin

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Received 26 September 2011; accepted 29 February 2012; published online 1 April 2012; doi:10.1038/ng.2228

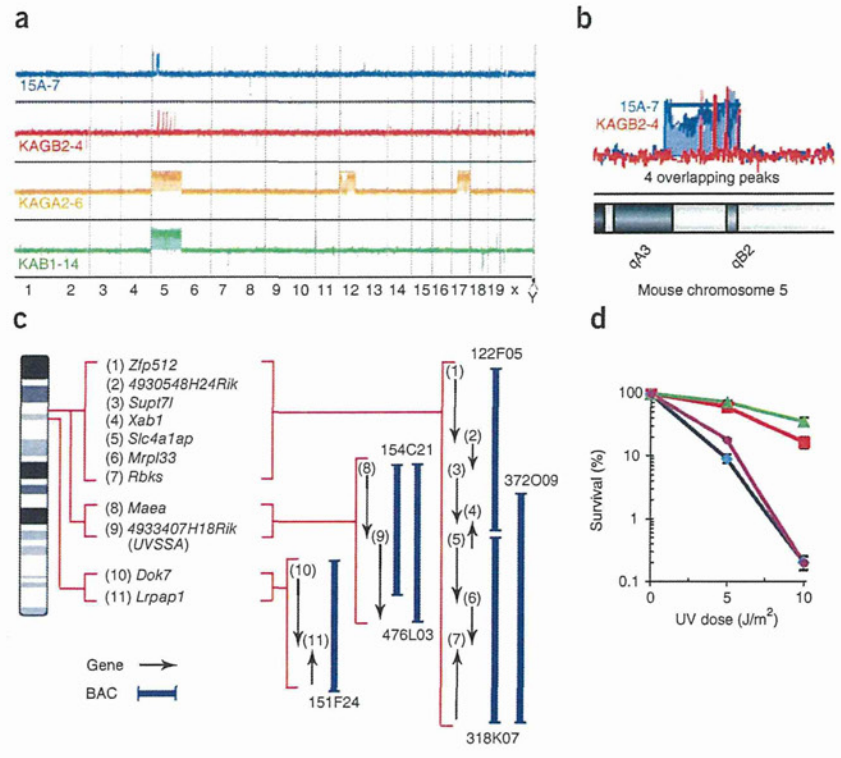

Figure 1 CGH array analysis and identification of BAC clones encompassing mouse genomic DNA integrated in the 15A-7 and KAGB2-4 clones. (**a**) CGH array analysis for identification of mouse genomic DNA integrated into the Kps3 clones containing mouse A9 chromosome segments. Kps3 cells were used as a control. (**b**) Enlarged view of the mouse chromosome 5 region integrated into the 15A-7 and KAGB2-4 clones. (**c**) Schematic of BAC clones encompassing the common 600-kb region of mouse genomic DNA integrated in clones 15A-7 and KAGB2-4. Genes included in the region are listed in positional order with a corresponding number. Chromosomal regions in red brackets correspond to peaks 2 to 4, from the left side, in **b**; there are no genes in peak 1. Arrows indicate the direction of the genes. (**d**) UV survival of Kps3 cells transfected with BAC 476L03 (green triangles) or BAC 154C21 (purple circles), compared to normal human WI38VA13 cells (red squares) and parental Kps3 cells (blue rhombuses). Error bars, s.d. calculated from three independent experiments.

fraction of extracts derived from Kps3 cells expressing Flag- and HA-tagged wild-type UVSSA. In the soluble fraction, ERCC8 was coimmunoprecipitated with UVSSA, irrespective of UV irradiation, but not with RNA Pol II or ERCC6 (**Fig. 3a**). In the chromatin fraction, ERCC6, ERCC8 and RNA Pol II were coimmunoprecipitated with UVSSA after UV irradiation (**Fig. 3b**).

Next, we searched for new TCR proteins that interact with UVSSA. The wild-type UVSSA protein fused with Flag and HA tags was stably expressed in HEK293 cells. Whole-cell extract was used for affinity purification of the UVSSA complex with antibodies to Flag and HA. Mass spectrometric analysis of the UVSSA complex identified USP7 as an interacting protein (**Supplementary Fig. 2**). USP7 is a ubiquitin-specific protease that recognizes and removes ubiquitin from proteins. Numerous proteins have been identified as potential substrates of USP7, including Mdm2, p53, claspin, Chfr and histone H2B[8,15]. To determine whether USP7 is involved in TCR, wild-type cells were treated with *USP7* small interfering RNA (siRNA) (**Fig. 3c**). *USP7* siRNA–treated cells showed decreased UV-RRS and UV survival, implicating USP7 in TCR (**Fig. 3d,e**). In the *USP7* siRNA–treated cells, not only USP7 protein levels, but also those of UVSSA, were decreased (**Figs. 3c** and **4a**). The amount of *UVSSA* mRNA was not affected in *USP7* siRNA–treated cells (**Supplementary Fig. 3**). Conversely, knockdown of *UVSSA* did not result in decreased USP7 protein levels (**Fig. 4a**), probably because of a much greater quantity of USP7 relative to UVSSA in the cells. The amount of ERCC8 protein was not affected in *USP7* siRNA–treated cells (**Fig. 4b**). These results indicate that UVSSA forms a stable complex with USP7 and transiently binds ERCC8. Also, the UVSSA-USP7 complex was coimmunoprecipitated with ERCC8, irrespective of UV irradiation in the soluble fraction, and it was coimmunoprecipitated with ERCC6, ERCC8 and RNA Pol II in the UV-damaged chromatin fraction (**Fig. 3a,b**). Coimmunoprecipitation was also detected in the cells expressing a normal level of *UVSSA* (**Supplementary Fig. 4**). These results indicate that the UVSSA-USP7 complex and ERCC8 are recruited to the sites of RNA Pol II and ERCC6 in the UV-damaged chromatin fraction. In the accompanying paper by Schwertman *et al.*, it was shown that both ERCC6 and UVSSA were recruited to the spot locally irradiated with 254-nm UV-C light, although the recruitment of ERCC6 and UVSSA was much weaker than that of the global genome-repair protein XPC[16]. These results suggest that only a small portion of cellular UVSSA is involved in TCR.

It is known that ERCC6 has an essential role in recruiting TCR factors to the stalled RNA Pol II complex[17,18]. To examine whether the binding of UVSSA, USP7 and ERCC8 to RNA Pol II is dependent on ERCC6, the Flag- and HA-tagged UVSSA protein was expressed in CS1AN (CS-B) cells, and immunoprecipitation of the cell extracts was performed using antibodies to Flag and HA. USP7 and ERCC8 were coimmunoprecipitated with UVSSA in the chromatin fraction, irrespective of UV irradiation, but RNA Pol II was not, even after UV irradiation (**Fig. 3f**). These results suggest that UVSSA, USP7 and ERCC8 are loaded onto RNA Pol II in an ERCC6-dependent manner.

Next, to examine whether the binding of UVSSA and USP7 to ERCC6 and RNA Pol II is dependent on ERCC8, the Flag- and HA-tagged UVSSA protein was expressed in CS3BE (CS-A) cells, and immunoprecipitation of the cell extracts was performed using antibodies to Flag and HA. USP7 was coimmunoprecipitated with UVSSA in the chromatin fraction, irrespective of UV irradiation, but RNA Pol II and ERCC6 were not coimmunoprecipitated, even after UV irradiation (**Fig. 3g**). These results suggest that ERCC8 is required for recruitment of the UVSSA-USP7 complex to ERCC6 and RNA Pol II in the UV-irradiated chromatin fraction. In the accompanying paper by Schwertman *et al.*, it was shown that GFP-UVSSA accumulated at sites of UV damage caused by a 266-nm UV-C laser in CS-A and CS-B cells, and a UV-independent interaction between hyperphosphorylated RNA Pol II (RNA Pol IIo) and UVSSA was detected in CS-B cells by chromatin immunoprecipitation (ChIP) using cross-linking reagent[16]. We assume that ERCC6 is required for stable integration of UVSSA, USP7, ERCC8 and RNA Pol II into a functional TCR complex.

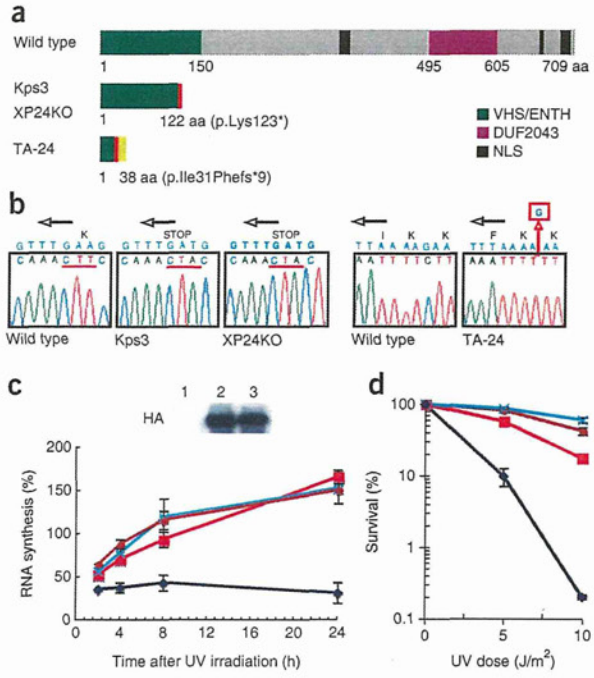

Figure 2 Mutations within the *UVSSA* gene in three subjects with UV^{S}S-A. (**a**) Schematic of the UVSSA protein and of amino-acid changes in Kps3, XP24KO and TA-24 cells. Amino-acid changes are shown in red. (**b**) Sequence chromatograms showing mutations in Kps3, XP24KO and TA-24 cells. The homozygous c.367A>T transversion is shown in Kps3 and XP24KO cells, and the homozygous c.87delG mutation is indicated in TA-24 cells. Translated amino acids are labeled above the cDNA sequence. Arrows represent the direction in which the sequence is read. (**c**) UV-RRS of Kps3 clones stably expressing *UVSSA* cDNA. Upper, protein blot of the Kps3 clones stably expressing Flag- and HA-tagged UVSSA using antibody to HA: lane 1, parental Kps3; lane 2, UVSSA-corrected Kps3 clone 1; lane 3, UVSSA-corrected Kps3 clone 2. Lower, UV-RRS of clones 1 (brown circles) and 2 (aqua crosses) and of WI38VA13 (red squares) and parental Kps3 cells (blue rhombuses). Error bars, s.d. calculated from three independent experiments. (**d**) UV survival of the Kps3 clones stably expressing Flag- and HA-tagged UVSSA. Symbols for each cell line are the same as in **c**. Error bars, s.d. calculated from three independent experiments.

We have found that the ERCC8 protein is translocated to the nuclear matrix after UV irradiation in an ERCC6- and transcription factor IIH (TFIIH)-dependent manner and that it colocalizes with Pol IIo[17,19]. Using a cell-free system[19], we found that UVSSA enhances UV-induced translocation of ERCC8 to the nuclear matrix (**Supplementary Fig. 5**).

It is known that inhibition of transcription after UV irradiation not only results from a blockage of transcription elongation by DNA damage on the transcribed strand but also can be caused by a reduction in the levels of the hypophosphorylated form of RNA polymerase II (RNA Pol IIa), which is required for the initiation of transcription[20]. The restoration of RNA Pol IIa levels and transcription after UV irradiation are deficient in CS-A and CS-B cells[20]. Consistent with this, we found that RNA Pol IIa disappeared after UV irradiation (10 J/m^2) in both the parental CS3BE (CS-A) cells and in ERCC8-corrected CS3BE cells. Approximately 16 h after UV irradiation, RNA Pol IIa reappeared in the ERCC8-corrected CS3BE cells but not in the parental CS3BE cells (**Supplementary Fig. 6**). We then examined the phosphorylation status of RNA Pol II in whole-cell lysates from Kps3 cells and UVSSA-corrected Kps3 cells after 10 J/m^2 of UV irradiation (**Fig. 4c**). RNA Pol IIa disappeared in both the parental and UVSSA-corrected Kps3 cells. However, it reappeared 8 h after UV irradiation in the UVSSA-corrected Kps3 cells but not in the parental Kps3 cells, indicating that UVSSA is required, in addition to ERCC6 and ERCC8, for the reappearance of RNA Pol IIa after UV irradiation.

Moreover, the amount of ERCC6 protein decreased in the non-irradiated parental Kps3 cells compared to the amount in the UVSSA-corrected Kps3 cells, and a greater decrease was detected after UV irradiation in the parental Kps3 cells, whereas there was little decrease in the UVSSA-corrected Kps3 cells (**Fig. 4c**). These results indicate

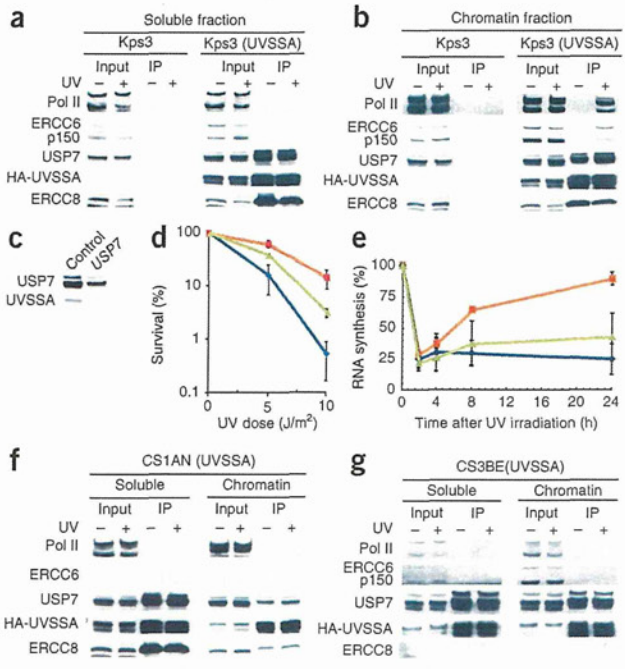

Figure 3 Interaction of UVSSA with RNA Pol II, ERCC6, ERCC8 and USP7. (**a**,**b**) Parental Kps3 cells and Flag- and HA-tagged wild-type UVSSA-corrected Kps3 cells were either irradiated with 20 J/m^2 of UV or were not irradiated and were incubated for 30 min. The UVSSA protein complex (IP) was then affinity purified from the soluble (**a**) or chromatin (**b**) fraction of each cell extract using anti-FLAG M2 agarose and anti-HA agarose and was subjected to protein blotting with antibodies to HA (UVSSA), RNA Pol II, ERCC6, ERCC8 and USP7. p150 corresponds to the CPFP protein (fusion protein consisting of N-terminal ERCC6$^{1-465}$ and the piggyback transposon)[32]. (**c**) Protein blot analysis of USP7 and UVSSA in UVSSA-corrected Kps3 cells and in isogenic cells transfected with *USP7* siRNA. The upper band of USP7 may be a modified form. (**d**) UV survival of parental Kps3 cells (blue rhombuses), UVSSA-corrected Kps3 cells (orange squares) and UVSSA-corrected Kps3 cells transfected with *USP7* siRNA (green triangles). Error bars, s.d. calculated from three independent experiments. (**e**) RNA synthesis after 10 J/m^2 of UV irradiation in the three cell lines in **d**. The symbols for each cell line are the same as in **d**. (**f**) Interaction of UVSSA with RNA Pol II, ERCC8 and USP7 in CS-B cells. Flag- and HA-tagged UVSSA protein was expressed in CS1AN (CS-B) cells, and immunoprecipitation of the cell extracts was performed using antibodies to Flag and HA. (**g**) Interaction of UVSSA with RNA Pol II, ERCC6 and USP7 in CS-A cells. Flag- and HA-tagged UVSSA protein was expressed in CS3BE (CS-A) cells, and immunoprecipitation of the cell extracts was performed using antibodies to Flag and HA. Error bars, s.d. calculated from three independent experiments.

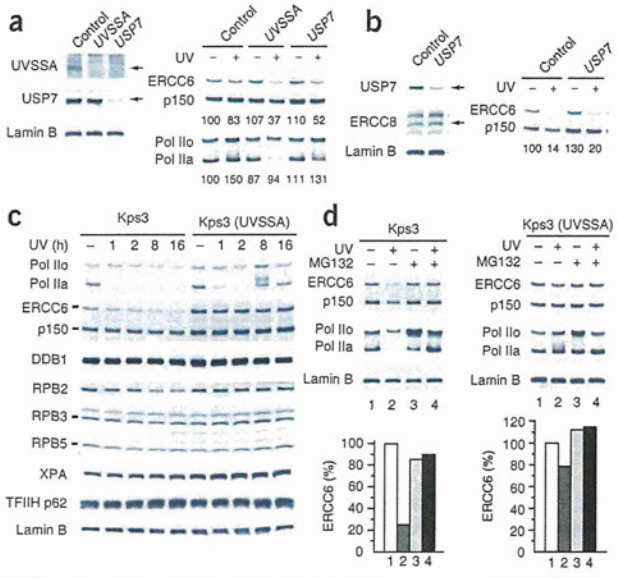

Figure 4 Degradation of ERCC6 in parental Kps3 cells, UVSSA-corrected Kps3 cells and normal cells transfected with *USP7* siRNA after UV irradiation. (**a**) UV-induced degradation of ERCC6 and RNA Pol IIo in normal cells transfected with control siRNA or siRNA targeting *UVSSA* or *USP7*. Knockdown of *UVSSA* and *USP7* was confirmed by protein blotting of cell lysates using the indicated antibodies. Normal cells transfected with each siRNA were irradiated with 10 J/m^2 of UV or not irradiated and were incubated for 16 h. Samples lysed with SDS gel-loading buffer were then analyzed by protein blotting with antibody to ERCC6. Numbers below the blots indicate the amounts of ERCC6 (upper) and RNA Pol IIo (lower) relative to their amounts in non-irradiated normal cells transfected with control siRNA. (**b**) UV-induced degradation of ERCC6 in Kps3 cells transfected with control siRNA or with siRNA targeting *UVSSA* or *USP7*. Knockdown by siRNA and protein blotting were performed as in **c**, except for an 8-h incubation after UV irradiation. Numbers below the blot indicate the amount of ERCC6 relative to the amount in non-irradiated Kps3 cells transfected with control siRNA. (**c**) Parental Kps3 cells and UVSSA-corrected Kps3 cells were irradiated with 10 J/m^2 of UV and incubated for the indicated times. Samples lysed with SDS gel-loading buffer were analyzed by protein blotting using the indicated antibodies. DDB1, UV-damaged DNA binding protein 1; RPB2, second largest subunit of RNA Pol II; RPB3, third largest subunit of RNA Pol II; RPB5, fifth largest subunit of RNA Pol II; XPA, xeroderma pigmentosum group A protein. Only RPB2 was decreased to some extent in UV-irradiated Kps3 cells. (**d**) Inhibition of the degradation of ERCC6 and the reappearance of RNA Pol IIa after UV irradiation in Kps3 cells in the presence of MG132. Parental Kps3 cells and UVSSA-corrected Kps3 cells were either irradiated with 10 J/m^2 of UV or not irradiated and were incubated for 8 h in the presence (10 μM) or absence of the proteasome inhibitor MG132. Samples lysed with SDS gel-loading buffer were analyzed by protein blotting using the indicated antibodies. Histograms indicate the amount of ERCC6 relative to the amount in non-irradiated cells in the absence of MG132.

that UVSSA has an important role in stabilizing ERCC6 in TCR. In addition, ERCC6 was degraded in the *USP7* siRNA–treated cells, as in the *UVSSA* siRNA–treated cells, but not in control siRNA–treated cells (**Fig. 4a**). Kps3 cells that lack *UVSSA* were then treated with *USP7* siRNA. No significant difference in UV-induced degradation of ERCC6 was detected between control siRNA– and *USP7* siRNA–treated Kps3 cells (**Fig. 4b**). These results indicate that UVSSA and USP7 cooperate to protect ERCC6 from UV-induced degradation in TCR.

When the Kps3 cells were UV irradiated in the presence of the proteasome inhibitor MG132, the reduction in ERCC6 levels after UV irradiation was suppressed, and RNA Pol IIa reappeared 16 h after UV irradiation (**Fig. 4d**). These results suggest that, in UV-irradiated Kps3 cells, ERCC6 is ubiquitinated and degraded by the ubiquitin-proteasome pathway, and the recovery of RNA Pol IIa is blocked, leading to the inhibition of UV-RRS.

The amount of RNA Pol IIo decreased in parental Kps3 cells (**Fig. 4c**) and in *UVSSA* siRNA–treated normal cells (**Fig. 4a**), although the decrease in RNA Pol IIo was not significant compared with that of ERCC6. RNA Pol IIo levels did not decrease in the UVSSA-corrected Kps3 cells and in control siRNA–treated cells (**Fig. 4a,c**). In the *USP7* siRNA–treated cells, the amount of RNA Pol IIo slightly decreased after UV irradiation when compared to the amount in control siRNA–treated cells (**Fig. 4a**). Taken together, these results suggest that UVSSA and USP7 are involved in the stabilization of RNA Pol IIo, but the effect might be indirect.

The affected individuals with the UV^{S}S, UV^{S}1KO and UV^{S}1VI, had homozygous mutations in the *ERCC6* and *ERCC8* genes, respectively[5,6]. This prompted us to examine whether some individuals with Cockayne syndrome features and TCR deficiency have mutations in the *UVSSA* gene. We sequenced the ORF of *UVSSA* in three TCR-deficient Cockayne syndrome cell lines (CS7099, CS6864 and CS2760) that have no mutations in the *ERCC6* and *ERCC8* genes. We found only SNPs in *UVSSA* in these cells (**Supplementary Fig. 7**), suggesting that there are some other gene(s) involved in the Cockayne syndrome phenotype beyond *ERCC6* and *ERCC8*. To exclude the possibility that some individuals with Cockayne syndrome have mutations in *UVSSA*, it is necessary to sequence the *UVSSA* ORF in many other TCR-deficient subjects with Cockayne syndrome.

It has been reported that CS-A and CS-B cells are hypersensitive to treatment with hydrogen peroxide and potassium bromate, specific inducers of oxidative DNA damage, and are deficient in the repair of oxidative DNA damage, whereas Kps3 (UV^{S}SA) and UV^{S}S1VI (UV^{S}S/CS-A) cells are not hypersensitive to oxidative DNA damage[6,21,22]. It has also been reported that transcription by RNA Pol II was reduced in the extracts of CS-A and CS-B cells compared to transcription levels in normal cells[23,24]. Nuclear extracts of CS-B cells failed to transcribe human rDNA, whereas those of CS-B cells expressing wild-type ERCC6 showed high transcriptional activity of RNA Pol I (ref. 25). Of note, RNA Pol I transcription was proficient in UV^{S}1KO (UV^{S}S/CS-B) cells[26]. Taken together, these results suggest that marked differences in the pathological phenotypes between Cockayne syndrome and UV^{S}S are caused by differences in transcription and/or in the repair of oxidative DNA damage in affected individuals.

We also determined that ERCC6 was ubiquitinated and degraded by the ubiquitin-proteasome pathway after UV irradiation in Kps3 cells (**Fig. 4c,d**). It has been reported that ERCC6 is required for the resumption of transcription after UV irradiation and for the recruitment of RNA Pol II and other transcription factors at the promoter in UV-irradiated cells[27]. It is therefore suggested that the ubiquitination and degradation of ERCC6 in the absence of UVSSA prevents recovery of RNA Pol IIa after UV irradiation and, consequently, blocks UV-RRS (**Supplementary Fig. 8**). Kps3 cells are deficient in the removal of UV damage on transcribed DNA strands[28], indicating that UVSSA is also required for excision of DNA damage in TCR (**Supplementary Fig. 8**).

It is not yet clear which E3 ligase is involved in the ubiquitination of ERCC6 in TCR. It would be interesting to examine whether ERCC6 is ubiquitinated by ERCC8 (ref. 29), BRCA1-BARD1 (ref. 30) or the p44 subunit of TFIIH[31]. UVSSA may negatively regulate E3 ligase activities. Alternatively or simultaneously, it may positively regulate