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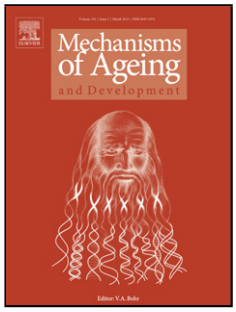

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Exosome-based immunomodulation during aging: a nano-perspective on inflamm-aging.

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Abstract

Exosomes are nanovesicles formed by inward budding of endosomal membranes. They exert complex immunomodulatory effects on target cells, acting both as antigen-presenting vesicles and as shuttles for packets of information like proteins, coding and non-coding RNA, and nuclear and mitochondrial DNA fragments. Albeit different, all such functions seem to be encompassed in the adaptive mechanism mediating the complex interactions of the organism with a variety of stressors, providing both for defense and for the evolution of symbiotic relationships with others organisms (gut microbiota, bacteria, and viruses). Intriguingly, the newly deciphered human virome and exosome biogenesis seem to share some physical-chemical characteristics and molecular mechanisms. Exosomes are involved in immune system recognition of self from non-self throughout life: they are therefore ideal candidate to modulate inflamm-aging, the chronic, systemic, age-related pro-inflammatory status, which influence the development/progression of the most common age-related diseases (ARDs). Not surprisingly, recent evidence has documented exosomal alteration during aging and in association with ARDs, even though data in this field are still limited.

Here, we review current knowledge on exosome-based trafficking between immune cells and self/non-self cells (i.e. the virome), sketching a nano-perspective on inflamm-aging and on the mechanisms involved in health maintenance throughout life.

Introduction

Eukaryotic cells release a variety of extracellular vesicles (EVs) that differ in content and biophysical properties. EVs can be divided into three main classes according to their biogenesis: shedding microvesicles, apoptotic bodies, and exosomes**.** Exosomes derived from intracellular organelles, or multivesicular bodies (MVBs), are released by several different eukaryotic cells. Two distinct MVB pathways lead to lysosomal targeting and exosome secretion, respectively; the latter pathway is characterized by MVB fusion with the plasma membrane **(Buschow et al., 2009).** Consequently, exosomes contain molecules derived both from endosomes, like major histocompatibility complex (MHC) class II, transferrin receptor, and clathrins, and from the cell surface, including different receptor types **(Willms et al., 2016).**

The finding that exosomes also contain nucleic acids, such as fragments of nuclear and mitochondrial DNA, and coding and non-coding RNAs, has been a revolutionary biomedical discovery **(Zhao et al., 2016; Huang et al., 2013; Silva and Melo, 2015; Thakur et al., 2014)**. Increasing evidence suggest that exosome-associated molecules reflect the pathophysiological status of releasing cells, and that their transfer may be an efficient way to induce metabolic changes in target cells **(de Jong et al., 2012; Kooijmans et al., 2016)**. In support to this hypothesis, it should be noted that much of the load carried by exosomes consists of molecules exerting epigenetic actions (i.e. modifications that do not induce changes in the genetic code but rather in its decoding). Such molecules include: i) molecules that act directly on DNA by promoting covalent (e.g. DNA methylases and demethylases) or non-covalent binding (e.g. protein transcription factors) **(Quian et al., 2015)**; (ii) agents that modulate DNA accessibility by promoting covalent binding to histones (e.g. histone methylases and acetylases); (iii) mRNAs that induce de novo protein synthesis in target cells; and (iv) microRNAs (miRNAs) and/or pre-miRNAs, which bind to mRNAs, modulating their translation **(Smythies and Edelstein, 2012; Melo at al., 2014)**.

Since information exchange between cells is closely controlled by the immune system, it is highly unlikely for immune system cells to be mere viewers in the intense EV trafficking. The recent discovery that exosomes from damaged cells, including those enriched with DNA damage markers such as γH2AX and fragmented telomere repeat DNA, are able to elicit an inflammatory response **(Wang and Lieberman, 2016)** supports the notion that exosome content can exert an influence on inflammatory responses. Recent evidence shows that exosomal miRNAs can also modulate cellular response to endotoxins **(Alexander et al., 2015),** and participate in the exchanging of antigens from bacteria, parasites, and virus-infected cells **(Marti and Johnson, 2016).** In addition, exosomal alterations have been described during aging and in patients with the most common age-related diseases (ARDs) **(Weilner et al., 2013; Xu and Tahara, 2013).** Overall, age-related modifications in exosome amount and content could affect the crosstalk between organs and tissues and the signaling between immune system cells and self/non-self cells. However, despite the potential relevance of this topic to human health, data are still scarce and inconclusive.

Here, we hypothesize that age-related alterations in exosome-mediated communication may be involved in the immune system inflammatory activation that is associated with aging, i.e. inflamm-aging **(Franceschi et al., 2000**) and in age-related remodeling of immune system activity, i.e. immunosenescence **(Fulop et al., 2015)**. Given their relevance to human health, the molecular mechanisms that link exosome trafficking to inflamm-aging and immune-senescence deserve further investigation.

Exosomal trafficking

A growing body of evidence suggests that exosomes transfer information not only in a paracrine fashion, but also through systemic mechanisms, providing a new pathway for the crosstalk among different cell types in a variety of pathophysiological conditions **(Ratajczak and Ratajczak, 2016**).

As regards homing, the exosome integrin pattern appears to be the main determinant of exosome tropism, as in the case of exosomes released by cancer cells **(Hoshino A 2015).** Surface receptors

on exosomes may act as identifying signals, as suggested for glycan **(Batista et al., 2011)** and heparin sulfate proteoglycan signatures **(Christianson et al., 2013).** Exosomes communicate with target cells both through cell membrane-mediated signaling and/or through internalization, which depending on cell type may be clathrin/caveole-mediated endocytosis, phagocytosis, or macropinocytosis **(Urbanelli et al., 2013).**

The exosome-based crosstalk relies on the ability of exosomes to transfer “packets of information” to recipient cells more efficiently than "single instructions", and in a more cost-effective way. Notably, since exosome-borne information reaches target cells in remote locations, rather like a "message in a bottle", the activities of exosomes on target cells are likely to be determined not only by the composition of their cargo, but also by the metabolic status of target cells **(Ohno et al., 2016)**.

Another function served by exosomes is to carry away harmful or unwanted material secreted by cells to preserve intracellular protein and RNA homeostasis **(Baixauli et al., 2014).** Notably, increased exosome release has been described in cells exposed to stress stimuli like glucose starvation and hypoxia, despite their unfavorable energetic status **(Garcia et al., 2015)**. The exosome cargo may also exert beneficial effects on stressed target cells, as demonstrated by recent evidence that cancer cells can exploit it as a source of nutrients in nutrient deprivation or nutrient-stressed conditions **(Zhao et al., 2016)**. Besides such extreme conditions, the functions exerted by exosomes are primarily related to the crosstalk among cells of different tissues, which necessarily involves immune system cells.

Exosomes as the interface between the virome and inflamm-aging

Even though exosomes are emerging as important constituents of the eukaryotic cell secretome, they seem to have been conserved during evolution, since bacteria and fungi also release microvesicles (**Mashburn and Whiteley, 2005; Deatherage and Cookson, 2012)**. Parasite-derived EVs can transfer virulence factors and drug resistance markers, alter host cell gene expression, and

promote parasite adherence and host cell proliferation **(Marti and Johnson, 2016).** Moreover, infected cells can secrete exosomes that contain pathogenic compounds, which thus deliver noxious cargo to distant organs and tissues (**Shimoda et al., 2016)**. Very recent data suggest that EVs are also a novel mode of viral propagation exploited by viruses to exit from cells non-lytically, to hide, and to manipulate the immune system **(Altan-Bonnet, 2016).** Several studies have documented a close similarity between exosome biogenesis, uptake, and secretion and the viral lifecycle molecular machinery **(van Dongen et a., 2016, Meckes DG Jr, 2015; Nolte-'t Hoen et al., 2016).** In keeping with their role as endogenous mRNA/miRNA carriers, EVs are exploited by viral RNA (e.g. human immunodeficiency virus and hepatitis C virus [HCV]) to propagate viral infection **(Chahar et al., 2015).** Indeed, EVs play a crucial role in HCV immune evasion, because serum-derived exosomes are infective and resistant to neutralizing antibodies **(Liu et al., 2014b).** Intriguingly, HCV-infected cells trigger an anti-viral response in neighboring infected cells, thus helping circumscribe and halt the infection**.** However, exosomes also play a pivotal role in some DNA virus infections, contributing to tumorigenesis, immune evasion, and viral latency **(Schwab et al, 2015).**

In particular, Epstein Barr virus (EBV) and herpes simplex virus 1 exploit exosomes both for spreading and/or curtailing viral mRNA and miRNA propagation throughout the organism **(Gutzeit et al., 2014: Han et al., 2016; Kalamvoki et al., 2014)**. Interestingly, latent EBV infection induces increased secretion of several inflammatory factors, whereas lytic infections evade the antiviral inflammatory response. A recently discovered molecular mechanism underpinning exosome uptake by receptor-ligand interactions with recipient cells has demonstrated specificity for cargo delivery, suggesting an intriguing overlap of exosomes and viruses (**van Dongen et a., 2016**). For instance, B cell–derived exosomes released from EBV-infected B cells can deliver their content to B cells, not to T cells **(Gutzeit et al., 2014).**

The virome behaves in a similar way to the microbiome, i.e. it arouses the immune system, suppresses inflammation, and occupies the ecological niche, crowding out potential pathogens. Like exogenous viruses, endogenous viral-like sequences (Alu, Line, Herv) are also virome constituents

(Virgin, 2014). High-throughput metagenomic sequencing analysis has demonstrated that hundreds of human and non-human viruses, mostly species that are not reported to be pathogenic in humans, outnumber their pathogenic counterparts and dwell in healthy individuals, contributing to the human virome **(Wylie et al., 2014).** Furthermore, recent literature reports that some viral species (i.e. papillomavirus, polyomavirus, and anellovirus) populate ecological niches paralleling the microbiome behavior **(Fancello et al., 2014).** For instance, unpublished data from our lab indicate that papillomavirus DNA is found in exosomes from circulating serum and urine also in the absence of overt disease, thus strengthening the hypothesis that exosomes are involved in the interplay between virome and immunosenescence.

Overall, EVs and exosomes seem to play important but opposing roles in viral disease pathogenesis, both as “Trojan horses” and as “marathon soldiers” **(Figure 1).**

Exosome-mediated crosstalk between immune cells

An effective immune response requires the engagement of host receptors by pathogen-derived molecules and the stimulation of appropriate cellular responses. Surprisingly, exosomes can shuttle pathogenic molecules that exert opposite functions, serving as antigens of innate immune receptors to activate host defenses or promoting pathogen immune evasion **(Schorey and Harding, 2016).** Exosomes therefore provide a new, complex system for communication among immune cells, i.e. T and B lymphocytes and antigen-presenting cells (APCs) that is to be added to the mechanisms involving receptor-ligand cell-cell interactions or receptor-soluble ligand interaction. Exosomes have well-characterized immune presentation properties that are related to their formation in MVBs, where the antigen is bound to the MHC molecules. Exosomes secreted by APCs, such as B cells, mast cells, and mature and immature dendritic cells (DCs), contain MHC class I and II molecules **(Pêche et al., 2006; Thery et al., 2001; Segura et al., 2005a)** and are able to bind to specific T cell receptors **(Eken et al., 2010)**; alternatively, they can be recaptured by DCs for antigen presentation **(Morelli et al., 2004).** Exosomes released from DCs have been shown to carry - besides peptide

MHC-I and -II - other T cell costimulatory molecules, such as CD80/B7.1 and CD86/B7.2, which exert a strong stimulation activity on the immune response **(Thery et al., 2002; 2009).**

DC-derived exosomes can also bind bacterial ligands for toll-like-receptor (TLR), thus acquiring the ability to induce strong activation of bystander DCs, enhancing proinflammatory cytokine secretion and stimulating the crosstalk with natural killer cells (**Sobo-Vujanovic et al., 2014).**

Thus, exosomes play a key role in disseminating pathogen- as well as host-derived molecules during infections **(Schorey et al., 2015).** Overall, DC-released exosomes are able to induce an immune response by spreading MHC-antigen complexes to both $CD4^+$ and $CD8^+$ T lymphocytes, or by spreading TLR ligands to other DCs (**Iraci et al., 2016)**. Adhesion molecules expressed on the exosome membrane, such as ICAM-1, can contribute to the interaction with lymphocyte function-associated antigen (LFA)-1 expressed by DCs and T lymphocytes (**Iraci et al., 2016).** Exosomes may stimulate immune system function also in allergic responses. DC-derived exosomes present allergenic antigens, inducing T cell T(H)2-like cytokine production in allergic donors **(Vallhov et al., 2015).** These findings suggest that such exosomes are involved in a potent mechanism that systemically alerts the host immune system to pathogen/allergen invasion.

The immune modulation action of exosomes includes not only antigen presentation, but also immune suppression **(Zhang et al., 2014).** Recent hypothesis suggest a role of exosomes to establish central tolerance, contributing to tissue-restricted antigen presentation within the thymic micromileus **(Skogberg et al., 2015).** When DC-derived exosomes are injected prior to skin graft implantation in animal models, exosomes from mature DCs can trigger effector T cell responses, leading to rapid graft rejection **(Segura et al., 2005b),** whereas exosomes from immature DCs inhibit anti-donor immune responses, significantly prolonging heart allograft survival **(Peche et al., 2003**). Moreover, oral administration of a protein antigen generates tolerosomes that induce tolerance when transferred to naive recipients **(Ostman et al., 2005).**

The contrasting immunomodulatory effects induced by exosomes, i.e. activation and inhibition of the immune response, may depend on the relative concentrations of specific subsets of antigen-

presenting exosomes and/or on the duration of exposure of immature immune cells to specific exosome subsets. The notion is strongly supported by data from in vitro and in vivo animal models, demonstrating the efficient alloantigen presentation and immunomodulatory abilities of exosomes in organ transplants **(Monguió-Tortajada et al., 2014).** Importantly, such activity could be directly mediated either by peptide-loaded MHC molecules or by accompanying epigenetic information, e.g. miRNAs, carried by antigen-loaded exosomes. For instance, exosomes derived from LPS-stimulated DCs contain sets of miRNAs that are capable of modulating endotoxin response in vivo **(Alexander et al., 2015).** Notably, exosomes containing miRNAs (i.e. miR-21) can directly activate intracellular TLR receptors, triggering a proinflammatory response **(Fabbri et al., 2012).**

A key notion in this context is that immune cell-derived exosomes can also modulate the phenotype of endothelial cells (ECs), thus promoting a systemic response **(Prattichizzo et al., 2016).** Monocyte-released exosomes can induce expression of adhesion molecules and cytokine secretion in ECs **(Tang et al., 2016)** or enhance their migratory properties **(Zhang et al., 2010)**. On the other hand, exosomes derived from cardiomyocytes can transfect ECs to deliver signals that mediate heart repair after injury **(Yuan et al., 2016).**

It is conceivable that the aging process, by tilting the balance toward a persistent proinflammatory state, may compromise exosome-based communications among cells and immune system function.

Age-related changes in the circulating exosome pool

Aging is a complex phenotype associated with a variety of molecular and tissue alterations. Recent data from anti-aging and regenerative research indicate that microenvironmental and circulating factors deserve close attention **(Almaça et al., 2014; Scudellari, 2015; Childs et al., 2015).**

Our group has been providing evidence that sets of circulating miRNAs involved in the modulation of inflammation (thus designated inflammamiRs) are deregulated in aging and in ARDs **(Olivieri et al., 2015).** Circulating miRNAs are either exosome-borne or protein-bound, and both types can be functionally transferred to recipient cells (**Turchinovich et al., 2015)**. The majority of miRNAs modulating inflammation, immunity, and aging pathways were seen to be exosome-associated

(Figure 2). These miRNA signatures are also involved in the modulation of insulin/IGF-1, mTOR, and other pathways relevant to aging and cellular senescence **(Olivieri et al., 2015).**

Accumulating senescent and/or pre-senescent cells during aging may influence the release and/or content of the circulating exosome pool. The progressive accrual of senescent cells during organismal aging could modulate the systemic exosome pool through two different mechanisms involving release by senescent cells of: i) a different amount of exosomes compared with younger cells, or ii) exosomes with a different content from exosomes derived from younger cells. The few available data support both hypotheses **(Weilner et al., 2013),** even though recent findings suggest that inflamm-aging and frailty do not result in an increased concentration of circulating EVs **(Alberro et al., 2016).** Evidence from cellular models suggests that exosomes released by senescent cells are more proinflammatory than those released by younger cells **(Mitsuhashi et al., 2013).** Moreover, even though plasma platelet-derived exosome number was lower in subjects older than 65 years than in younger ones, chemokine and HMGB1 levels were higher in the former subjects **(Goetzl et al., 2016).** These data support the hypothesis that macrophages and platelet-derived exosomes may contribute to the systemic spread of inflamm-aging. Interestingly, reduced phagocytic activity has been reported in monocytes from elderly donors both in mice and humans, demonstrating dysregulation of monocyte subpopulations with age **(Bliederhaeuser et al., 2016).**

Significantly increased exosome release has been described in prostate cancer cells exposed to high **c**linical doses of radiation, which is a powerful mechanism inducing senescence through p53 activation **(Lehmann et al., 2008).** Interestingly, p53 pathway influences exosome formation in colorectal cancer cell lines **(Sun et al., 2016)** and an increased microvesicles shedding induced by pro-senescence stimuli was observed also in non-cancerous cell lines **(Effenberger et al., 2014)**.

These data lend support to the hypothesis that cellular senescence induced either by telomere attrition (i.e. replicative senescence) or by DNA damage (i.e. radiotherapy) may induce a p53-dependent increase in the biogenesis of exosome-like vesicles and/or alter their cargo. Notably, senescence is associated with the acquisition of a proinflammatory and secretory phenotype -

designated SASP (senescence associated secretory phenotype) **(Lecot et al., 2016)** or SMS (senescence messaging secretome) **(Kuilman and Peeper, 2009)** - that promotes tumor progression (mostly by influencing the tumor-immune system crosstalk) and chemoresistance **(Lecot et al., 2016; Di Mitri and Alimonti, 2016)**, and accelerates aging **(Childs et al., 2015).** Interestingly, p53 reactivation in advanced liver cancer has been associated with a functional SASP that was capable of attracting innate immune cells (neutrophils, macrophages, and natural killer cells), thus promoting the clearance of pre-malignant cells **(Xue et al., 2007).** Moreover, NF-kB, the main pro-inflammatory transcription factor and key SASP modulator **(Salminen et al., 2012),** has recently been involved in the modulation of exosome cargo. NF-kB -/- mice show an altered exosome content after exposure to a noxious stimulus **(Yang et al., 2015).** Furthermore, the SASP can transmit senescence to bystander cells **(Acosta et al., 2013).** Latent EBV infection induces increased secretion of several inflammatory factors, whereas lytic infections evade the antiviral inflammatory response. Interestingly, latent EBV infection in EBV-positive cells induces senescence and SASP acquisition in neighboring ECs, whereas lytic EBV infection abolishes this phenotype through downregulation of TNF-α secretion and consequent suppression of senescence transmission **(Long et al., 2016).** Moreover, EBV viral oncogene latent membrane protein 1 (LMP1) promotes endosomal-exosomal pathway trafficking, which in turn suppresses NF-kB activation **(Verweij et al., 2011).** These data sustain the hypothesis that viruses exploit evolutionarily conserved and aging-associated mechanisms to support their spread and/or survival, and that exosomal trafficking has a central role in this interplay.

Notably, senescent cells show lysosomal enzyme deregulation, i.e. increased β-galactosidase activity. It has been hypothesized that lysosome malfunction could be compensated for through release of potentially toxic cargo into EVs **(Eitan et al., 2016).** Interestingly, strong SASP suppression by rapamycin is associated with decreased β-galactosidase expression without cell cycle re-entry (**Laberge et al., 2015**), indicating that lysosomal β-galactosidase is a marker of secretory activity, rather than of cell cycle arrest (**Serrano 2015**).

Overall, a greater understanding of MVB trafficking to lysosomes and the plasma membrane is expected to provide insight into diseases where pathogenic proteins, lipids, or infectious agents accumulate in or outside cells **(Eitan et al., 2016).** Release by senescent cells of exosomes with abnormal cargo can exert different effects on cells living in a youthful or an aged milieu. It has been reported that peripheral exosome-mediated delivery of miRNAs from a youthful systemic milieu enhanced myelination in aging brain, mimicking the effect of youth on CNS myelination **(Pusic and Kraig, 2014).** In this regard, parabiosis experiments that connected the circulation of an aged and a young mouse have achieved a rejuvenation effect in several tissues of the aged mouse (**Horrington et al., 1960, Scudellari, 2015**). However, all efforts to identify the molecular agents exerting the rejuvenation effects in the young animal's plasma have not yet met with success **(Reardon, 2015)**. Finally, lysosomes have been reported to contain molecules that increase *Caenorhabditis elegans* lifespan **(Folick et al., 2015).**

Exosomes and circulating inflammamiRs

It is still unclear whether exosome-associated non-coding RNA, such as miRNAs and long non–coding RNAs, is merely representative of the cell of origin or whether selective loading occurs **(Zhang et al., 2015b)**. Evidence has been provided that a subset of miRNAs preferentially enter exosomes **(Guduric-Fuchs et al., 2012)** and that some miRNAs are overrepresented in exosomes compared with the cell of origin **(Goldie et al., 2014).** Neural sphingomyelinase 2 (nSMase2), sumoylated heterogeneous nuclear ribonucleoproteins (hnRNPs), the 3′-end of the miRNA sequence, and miRNA-induced silencing complex (miRISC) all appear to be key players in miRNA loading into exosomes **(Zhang et al., 2015b).**

However, since aging is a complex phenotype involving multiple tissues and organs, identifying a shared subset of miRNAs, i.e. inflammamiRs **(Olivieri et al., 2015)**, capable of regulating the main age-related processes and pathways could be decisive to gain insights into organismal aging. Based on this hypothesis, we have combined all available data on the miRNAs relevant to aging,

inflammation, and immunity, focusing on those that have been demonstrated to be carried by exosomes. The results are depicted in **Figure 2.** The panel of miRNAs common to the three pathways includes some important inflammamiRs, i.e. miR-19b, -20a, -21, -126, -146a, and -155. A list of the chief targets of these inflammamiRs is reported in **table 1.** DNA damage response, oxidative stress, proteotoxic stress, mitochondrial dysfunction, senescence, inflamm-aging, and nutrient sensing pathways are all directly or indirectly affected by this miRNA panel, suggesting a key role for them in organismal aging.

Notably all the miRNAs in this panel share common features: i) an altered expression in senescent cells and/or in plasma/serum or in microparticles/exosomes from patients with the major ARDs; ii) the ability to modulate inflammatory pathways, and iii) the capacity to modulate bacterial and/or viral infections in immune cells **(Oliveri et al., 2015).**

In previous papers we have reported that the circulating plasma levels of miR-21 and miR-126 increase during aging in healthy subjects **(Olivieri et al., 2012; Olivieri et al., 2014).** Moreover, we have described altered circulating levels of these miRNAs in a variety of ARDs **(Olivieri et al., 2015).** Since circulating miRNA levels are affected by a variety of factors, they do not yet have clinical diagnostic/prognostic value for ARDs **(Prattichizzo et al., 2015)**. In this context, exosomes could provide a more accurate source of miRNA-related information. Indeed, a recent pilot study has suggested a salivary exosomal miR, miR-24-3p, as a candidate biomarker of aging **(Machida et al., 2015).** Moreover, emerging evidence highlight the relevance of secreted and microvesicles contained miRNAs in tissues crosstalk, in the context of ARDs. Endothelial-miR-31 can be secreted by senescent cells inside MVs and can be taken-up by mesenchymal stem cells, inhibiting osteogenic differentiation by knocking down its target Frizzled-3 (**Weilner et al., 2016**). Further, miR-31 circulating levels increase during aging especially in osteoporotic patients, providing a proof of principle of the relevance of exosomal miRNAs in fostering a pro-aging environment (**Weilner et al., 2016).**

Future perspectives

Aging is not a static, but rather a dynamic phenotype resulting from continuous interactions between genetic make-up and environmental factors, including the metabioma harbored in almost all body compartments. Since it involves the entire organism, it is conceivable that health preservation during aging is ensured by efficient tissue and organ crosstalk modulating the changes induced by such interactions. Based on this hypothesis, age-related health deterioration would stem from defective tissue and organ crosstalk, with molecules circulating in the bloodstream being the main culprits. What if the circulating factors that have been identified as modulators of the aging process were “hidden” inside exosomes? Exosomes transport packets of information, rather than a single instruction, that exert a synergistic effect on target cells. Among these quanta of information, there are those produced by the inhabitants of the healthy body, especially the virome constituents. Chronic exosome parabiosis experiments, like repeated administration to an old mouse of exosomes from a healthy young mouse, could shed light on this intriguing question. Future studies are required unravel the molecular mechanisms underpinning exosome modulation during aging and the effects of such deregulation on inflamm-aging/immunosenescence, to pave the way for innovative strategies, including virome manipulation, directed at slowing down inflamm-aging and postponing ARD development.

Competing interests

The authors declare that they have no competing interests.

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Table 1. mRNA targets and related pathways modulated by the exosome-carried miRs modulating Inflammation, Immunity and Aging

Figure 1. Exosomes can play opposing roles in viral disease pathogenesis

Figure 2. Venn diagram displaying the miRs related to Inflammation, Immunity, and Aging based on their circulating shuttles

Inner circles: exosome-associated miRs; Outer circles: circulating miRs associated with Ago-2, HDL, or other microparticles. Bold characters: miRs modulating at least two pathways

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Table 1. mRNA targets and related pathways modulated by the exosome-carried miRs modulating Inflammation, Immunity and Aging

MiRNA	mRNA targets	Related pathways	References
19b	BACE1, PTEN, TGFB-R2, TLR-2.	Proteostasis, tumor biology, senescence, SASP, immune cytokine signaling, response	Hébert et al., 2008; Liu et al., 2014; Mestdagh et al., 2010; Philippe et al., 2012.

		signaling, response	
20a	E2F1, E2F3, CDKN1A (p21), Stat3, TGFB-R2, PTEN, MAP3K12, MAP2K3.	Senescence, SASP, cytokine signaling, tumor biology, immune response	Sylvestre et al., 2007; Hackl et al., 2010; Zhang et al., 2011; Zhou et al., 2015; Zhang et al., 2015c; Pin et al., 2012; Beveridge et al., 2009.
21	IL-1B, ICAM1, SOD3, BCL2, TGFBR-2, TGFB-I, E2F1, PTEN.	Inflammasome, senescence, SASP, cytokine signaling, immune response, oxidative stress, tumor biology	Terao et al., 2011; Zhang et al., 2012; Si et al., 2007; Gabriely et al., 2008; Bhat-Nakshatri et al., 2009; Meng et al., 2007.
126	SPRED1, TOM1, VCAM1, IRS1, DNMT1, KRAS, VEGFA, IGFBP2, MMP7, CXCL12.	Senescence, SASP, cytokine signaling, immune response, insulin signaling, angiogenesis, epigenetic regulation, tumor biology	Fish et al., 2008; Oglesby et al., 2010; Zhang et al., 2008; Harris et al., 2008; Zhao et al., 2011; Jiao et al., 2012; Liu et al., 2009; Png et al., 2012; Felli et al., 2013; Zhang et al., 2013b.
146a	CXCR4, TLR-2, TLR-4, TRAF6, IRAK1, NFKB1, KIT, STAT1.	Senescence, SASP, cytokine signaling, immune response, tumor biology	Labbaye et al., 2008; Jurkin et al., 2010; Taganov et al., 2006; Bhaumik et al., 2008; Yang et al., 2011; Wang et al., 2013.
155	FOXO3a, TP53INP1, IKBKE, SMAD5, SOCS1, TRF1.	Telomere function, senescence, cytokine signaling, immune response, tumor biology	Kong et al., 2010; Gironella et al., 2007; Gibcus et al., 2009; Yin et al., 2008; Jiang et al., 2010; Dinami et al., 2014.

Hightlights

Exosomes are involved in immune system recognition

Exosomal alteration was observed during aging and age-related diseases

Exosomes can play opposing roles in viral disease pathogenesis

MicroRNAs related to Inflammation, Immunity and Aging are exosomes-associated

Exosomes can contribute to inflammaging

Figure 1

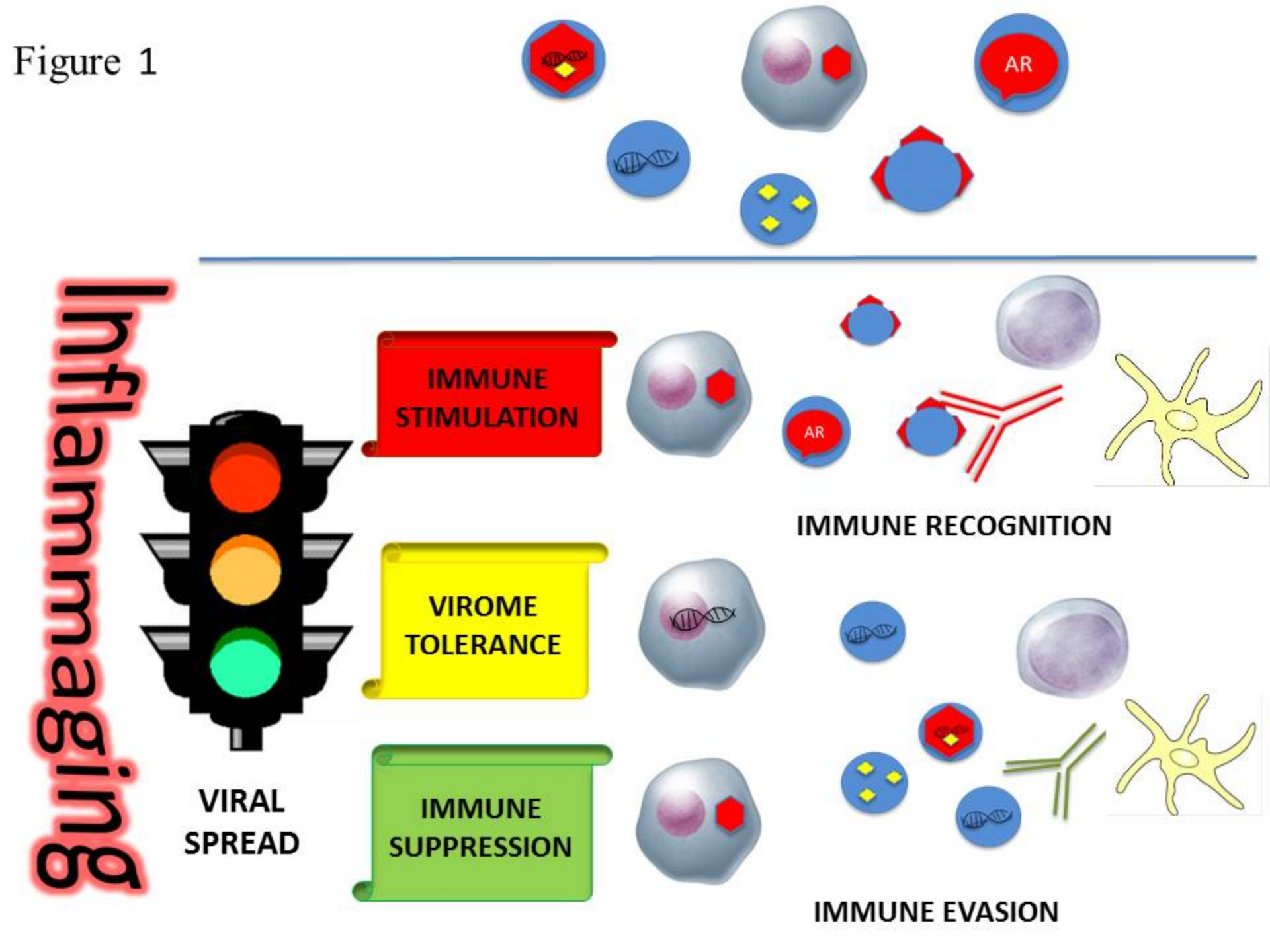
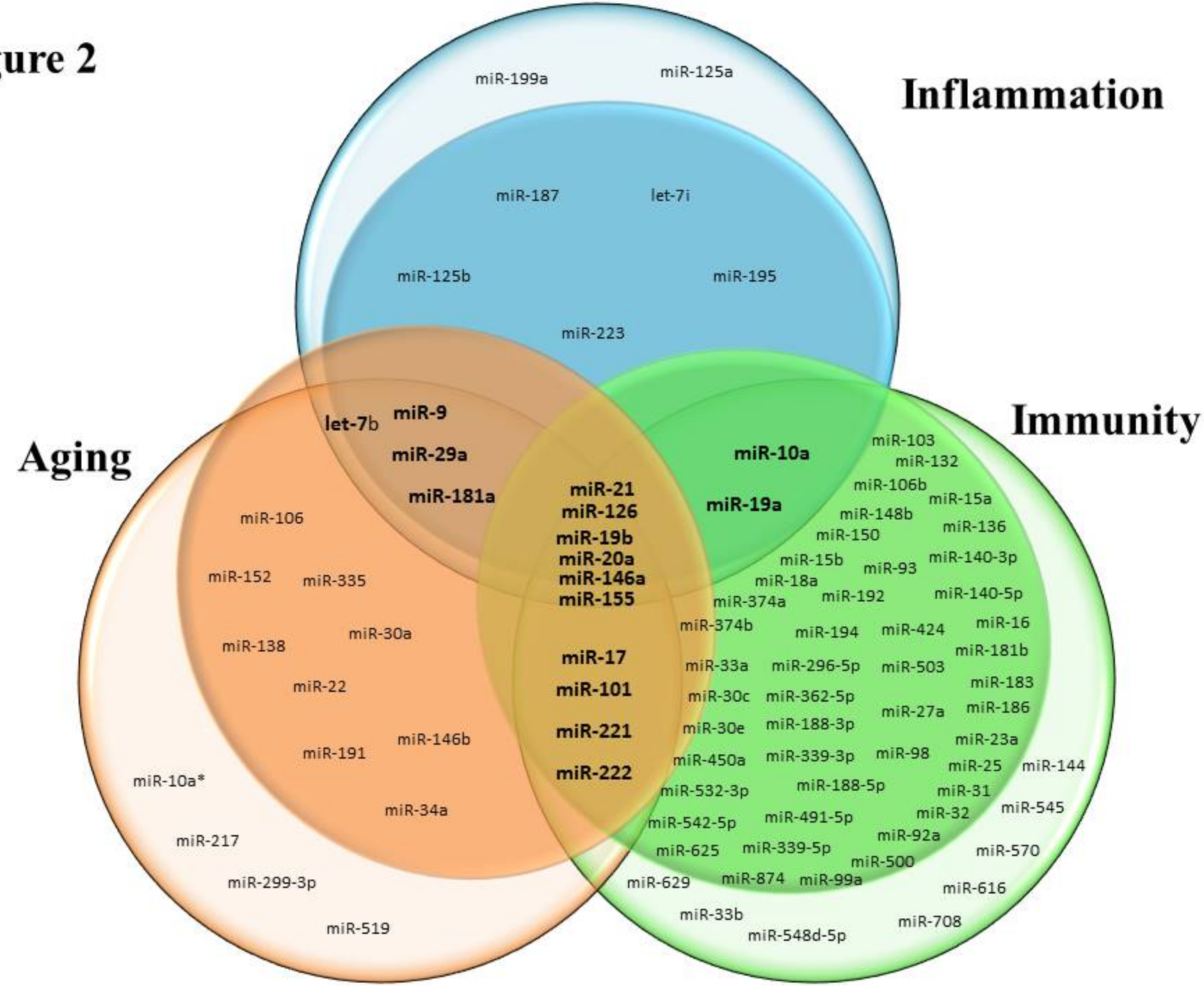

AR
IMMUNE STIMULATION
AR
IMMUNE RECOGNITION
VIROME TOLERANCE
VIRAL SPREAD
IMMUNE SUPPRESSION
IMMUNE EVASION
Inflammaging

Figure 2


Inflammation
Aging
Immunity
miR-199a
miR-125a
miR-187
let-7i
miR-125b
miR-195
miR-223
let-7b
miR-9
miR-29a
miR-181a
miR-106
miR-152
miR-335
miR-138
miR-30a
miR-22
miR-191
miR-146b
miR-10a*
miR-34a
miR-217
miR-299-3p
miR-519
miR-21
miR-126
miR-19b
miR-20a
miR-146a
miR-155
miR-17
miR-101
miR-221
miR-222
miR-10a
miR-19a
miR-103
miR-132
miR-106b
miR-15a
miR-148b
miR-136
miR-150
miR-15b
miR-93
miR-140-3p
miR-18a
miR-374a
miR-192
miR-140-5p
miR-374b
miR-194
miR-424
miR-16
miR-181b
miR-33a
miR-296-5p
miR-503
miR-183
miR-30c
miR-362-5p
miR-27a
miR-186
miR-30e
miR-188-3p
miR-23a
miR-450a
miR-339-3p
miR-98
miR-25
miR-144
miR-532-3p
miR-188-5p
miR-31
miR-542-5p
miR-491-5p
miR-32
miR-545
miR-92a
miR-625
miR-339-5p
miR-570
miR-500
miR-629
miR-874
miR-99a
miR-616
miR-33b
miR-708
miR-548d-5p