

外泌体在眼科疾病中的研究进展

罗家伟, 张国伟, 康丽华, 管怀进

引用: 罗家伟, 张国伟, 康丽华, 等. 外泌体在眼科疾病中的研究进展. 国际眼科杂志 2021;21(5):805-809

基金项目: 国家自然科学基金资助项目 (No.81974129)

作者单位: (226001) 中国江苏省南通市, 南通大学附属医院眼科研究所

作者简介: 罗家伟, 南通大学在读博士研究生, 住院医师, 研究方向: 白内障。

通讯作者: 管怀进, 毕业于中山大学, 教授, 主任医师, 博士研究生导师, 研究方向: 白内障与防盲治盲、眼分子生物学. guanhjeye@163.com

收稿日期: 2020-03-27 修回日期: 2021-03-26

摘要

外泌体(exosomes)是一类直径50~150nm的细胞外膜泡,可以递送生物活性分子(如蛋白质、脂质、DNA、miRNA等)至靶细胞中,以发挥细胞间通讯作用。外泌体介导的细胞间通讯影响靶细胞的凋亡、侵袭、迁移、免疫应答及氧化损伤修复等功能。近年来外泌体研究在眼科学领域迅速开展,本文总结了在眼科疾病中外泌体相关研究的最新进展。

关键词: 外泌体; 眼科疾病; 分子病理; 细胞间通讯; 研究进展

DOI:10.3980/j.issn.1672-5123.2021.5.11

Research progress on the exosomes in ophthalmic diseases

Jia-Wei Luo, Guo-Wei Zhang, Li-Hua Kang, Huai-Jin Guan

Foundation item: National Natural Science Foundation of China (No.81974129)

Eye Institute, the Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu Province, China

Correspondence to: Huai-Jin Guan. Eye Institute, the Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu Province, China. guanhjeye@163.com

Received: 2020-03-27 Accepted: 2021-03-26

Abstract

• Exosomes are extracellular vesicles of sizes ranging from 50-150nm in diameter. Exosomes can deliver bioactive molecules (e.g. proteins, lipids, DNA, microRNA, etc.) into target cells which play an important role in cell-cell communication. Researches demonstrated that exosomes mediated cell-cell communication can impact cell apoptosis, invasion, migration, immune response and oxidative repair ability of recipient cells. Recently,

researches on exosomes developed rapidly in the field of ophthalmology. This review summarized the latest research progress of exosomes in ophthalmic diseases.

• **KEYWORDS:** exosomes; ophthalmic diseases; molecular pathology; intercellular communication; research progress

Citation: Luo JW, Zhang GW, Kang LH, et al. Research progress on the exosomes in ophthalmic diseases. *Guoji Yanke Zazhi (Int Eye Sci)* 2021;21(5):805-809

0 引言

外泌体(exosomes)是一类直径50~150nm的细胞外囊泡,主要起源于细胞质内的多泡小体(multivesicular bodies, MVBs)^[1]。外泌体的分泌受神经酰胺^[2]、钙离子浓度^[3]、Rab蛋白^[4]等多因素调控。外泌体携有蛋白质、脂质、信使核糖核酸(messenger ribonucleic acid, mRNA)、微小核糖核酸(micro ribonucleic acid, miRNA)和脱氧核糖核酸(deoxyribonucleic acid, DNA)等多种生物活性分子,并能转运至靶细胞中,调控靶细胞的功能^[5]。外泌体与多种眼科疾病的发生发展相关,本文总结了外泌体在角膜损伤、糖尿病视网膜病变(diabetic retinopathy, DR)、年龄相关性白内障(age-related cataract, ARC)、青光眼、年龄相关性黄斑变性(age-related macular degeneration, ARMD)及葡萄膜黑色素瘤(uveal melanoma, UM)中的研究进展。

1 外泌体概述

1.1 外泌体的起源 细胞内组分可通过依赖转运必需内体分选复合物(endosomal sorting complexes required for transport, ESCRT)的途径或不依赖ESCRT的途径被特异性地转运进细胞内体中^[5],形成含有许多管腔内膜泡(intraluminal vesicles, ILVs)的内体,即MVBs。当MVBs膜与细胞膜融合时,这些ILVs被释放到细胞外,即为外泌体^[6]。

1.2 外泌体的提取方法 针对外泌体的密度、尺寸和表面抗原等特征,有多种提取外泌体的方法,如梯度超速离心法、聚乙烯二醇共沉淀法、密度梯度超速离心法、尺寸排阻色谱法、免疫捕获法等^[7]。上述方法在外泌体的得率和纯度上各不相同。针对不同的实验目的和样本类型,需选择适当的提取方法。

1.3 外泌体的特征 大部分外泌体共同携带的蛋白^[8]包括四跨膜蛋白超家族(如CD63、CD9、CD81)、热休克蛋白家族(如热休克蛋白70、热激同源蛋白70)、ESCRT相关蛋白(如Alix、TSG101),上述蛋白常被选作鉴定外泌体的特异性标志物。透射电子显微镜可观察到外泌体呈直径50~150nm,且周边隆起中央凹陷的圆形“茶托样”形态^[9]。纳米粒子追踪分析技术常用于分析样品中外泌体的尺寸分布和密度^[9]。

2 外泌体与眼科疾病

外泌体可在泪液^[10]、房水^[11]、玻璃体液^[12]和血液^[13]等眼部体液中稳定存在,在调节眼部细胞迁移^[14]、增生、凋亡^[15]、免疫反应^[16]和血管生成^[17]等方面发挥重要作用。外泌体与角膜损伤、DR、ARC、青光眼、ARMD及UM等眼病关系密切。

2.1 外泌体与角膜损伤 角膜的透明和完整对视力至关重要。角膜损伤后,需要及时愈合以防眼内感染。角膜愈合涉及凋亡、增殖、分化、迁移和细胞外基质重塑等多个生物学过程^[18]。新生血管形成是角膜愈合中常见的并发症。基质金属蛋白酶(matrix metalloproteinase, MMP)家族是一类促血管生成因子。MMP-14和MMP-2可重塑血管内皮细胞外基质,促进新生血管生长^[19-20]。血管内皮生长因子受体1(vascular endothelial growth factor receptor 1, VEGFR1)作为诱饵受体竞争性结合血管内皮生长因子A(vascular endothelial growth factor A, VEGFA),负调节其促血管生成作用^[21]。MMP-14可降解细胞表面的VEGFR1,从而解除对VEGFA的抑制,促进血管内皮细胞增殖和迁移,诱导血管形成^[14]。角膜成纤维细胞可通过外泌体将MMP-14递送至血管内皮细胞,以促进角膜新生血管的生成^[22]。除了角膜成纤维细胞外泌体,角膜上皮细胞外泌体也参与调控角膜愈合。Han等^[23]使用透射电镜在大鼠角膜损伤模型的角膜上皮细胞表面和角膜基质中观察到外泌体。小鼠角膜上皮细胞的外泌体可促进小鼠角膜成纤维细胞的增殖和分化,同时能促进人脐静脉血管内皮细胞(human umbilical vein endothelial cells, HUVECs)的增殖^[23]。Zieske等^[24]在体外构建了兔角膜后弹力层(含角膜内皮细胞)与角膜成纤维细胞三维共培养模型,并在后弹力层基质中与角膜成纤维细胞表面观察到外泌体。提示在角膜外伤愈合过程中角膜成纤维细胞、角膜内皮细胞、角膜血管之间存在潜在的外泌体介导的跨细胞通讯方式,但机制及意义尚未完全阐明。

间充质干细胞衍生的外泌体也可调控角膜愈合。Shen等^[25]报道,脂肪间充质干细胞(adipose-derived mesenchymal stem cell, ADSC)分泌的外泌体可促进角膜基质细胞的增殖、抑制其凋亡,且促进角膜基质细胞转化为角膜成纤维细胞。ADSC来源的外泌体还可抑制角膜基质细胞中MMP的表达,诱导细胞外基质相关蛋白(如胶原蛋白、纤维连接蛋白等)的表达,促进细胞外基质的合成以抑制新生血管。Tao等^[26]使用人胎盘来源间充质干细胞(human placenta-derived MSCs, hP-MSCs)分泌的外泌体孵育小鼠角膜碱烧伤伤口,发现hP-MSCs外泌体处理组角膜新生血管较对照组(PBS处理组)少,且透明性也优于对照组,角膜中促血管生成相关基因与炎症因子基因表达亦显著降低。Shojaati等^[27]报道人角膜基质来源的间充质干细胞(mesenchymal stem cells from corneal stromal stem cells, CSSC)分泌的外泌体也可抑制小鼠角膜伤口的瘢痕形成并维持角膜透明性,且发现CSSC外泌体的保护作用可能是通过递送miRNA实现的^[27]。上述结果均揭示了间充质干细胞外泌体对角膜愈合的保护性作用,但具体机制及临床应用价值仍有待研究。

2.2 外泌体与糖尿病视网膜病变 DR的基本病理为视网膜微血管病变,最终形成视网膜新生血管^[28]。新生血管

的形成与视网膜色素上皮(retinal pigment epithelium, RPE)通透性有关。病理状态的RPE通透性增加,未成熟的脉络膜新生血管(choroidal neovascularization, CNV)可穿透RPE生长到视网膜中,导致视力损害。

视网膜毛细血管主要由血管周细胞与内皮细胞组成。Liu等^[29]发现在糖尿病小鼠视网膜血管周细胞内环状RNA-cPWWP2A(circular RNA cPWWP2A, circRNA-cPWWP2A)表达上调,而在血管内皮细胞中表达无变化。血管周细胞中高表达的circRNA-cPWWP2A可抑制细胞凋亡,并通过外泌体以旁分泌的形式传递给血管内皮细胞。在内皮细胞中, circRNA-cPWWP2A作为分子海绵吸附miR-579,诱导血管生成素1、紧密连接蛋白、沉默信息调节因子1表达上调,增强血管内皮细胞的增殖、迁移和成管能力。外泌体circRNA-cPWWP2A介导的血管周细胞-内皮细胞跨细胞调控可能是视网膜毛细血管在高糖环境下对抗损伤和渗漏的代偿机制,但高糖环境诱导circRNA-cPWWP2A上调的机制依然不明。

除了血管周细胞外泌体,视网膜星形胶质细胞外泌体也在DR中发挥保护作用。在激光诱导的小鼠CNV模型中,视网膜星形胶质细胞分泌的外泌体可抑制巨噬细胞的迁移和血管内皮细胞成管功能,减轻视网膜血管炎症和渗漏^[17]。在视网膜星形胶质细胞外泌体中发现有12种抗血管生成因子(如内皮他丁)表达。抑制视网膜星形胶质细胞的内皮他丁表达后,其分泌的外泌体的抗视网膜血管渗漏作用消失^[17]。提示视网膜星形胶质细胞外泌体可能是通过递送抗血管生成因子来发挥保护作用的。

血-视网膜屏障的完整对避免DR中的视网膜血管渗漏至关重要。视网膜内皮细胞是组成血-视网膜屏障的重要部分。Zhang等^[30]发现,糖尿病大鼠血浆中血小板衍生的外泌体显著增加,且血小板外泌体中CXC趋化因子配体10(CXC chemokine ligand 10, CXCL10)也显著上调。糖尿病大鼠的血小板外泌体可向视网膜内皮细胞递送CXCL10激活TLR4信号通路,抑制超氧化物歧化酶活性,诱导活性氧产生和氧化损伤,破坏血-视网膜屏障。而体外构建的高表达miR-126的人间充质干细胞外泌体,可向视网膜内皮细胞传递miR-126抑制高迁移率族蛋白B1信号通路,减轻高血糖诱导的视网膜炎^[31]。上述研究结果为DR治疗提供了新思路。

过氧化物酶体增殖子激活受体 γ (peroxisome proliferators-activated receptors γ , PPAR γ)是一种促血管生成因子,它可诱导血管内皮生长因子(vascular endothelial growth factor, VEGF)上调,刺激新生血管形成。在增殖性DR患者的房水、玻璃体液和增殖膜中PPAR γ 表达上调,且表达量与视网膜纤维增生程度正相关^[12]。HUVECs分泌的外泌体也含有PPAR γ 。提示PPAR γ 可能由血管内皮细胞经外泌体释放到房水和玻璃体液中,调控新生血管生成。色素上皮衍生因子(pigment epithelium-derived factor, PEDF)具有抗血管生成作用^[32]。RPE细胞可定向地从顶端分泌携带PEDF的外泌体,而基底侧几乎不分泌^[33]。提示RPE衍生的外泌体可能在DR中发挥抗新生血管作用,但具体机制依旧不明。

2.3 外泌体与年龄相关性白内障 ARC是全球首位致盲性眼病,表现遗传调控是ARC重要的影响因素。miRNA

在 ARC 患者与晶状体透明的正常对照者所捐献眼球的晶状体上皮细胞中差异表达^[34-35],通过调控晶状体上皮细胞靶基因参与 ARC 的病程^[36]。Dismuke 等^[11]从 ARC 患者房水外泌体中检测出多种 miRNA,推测房水外泌体可能通过递送 miRNA 调控晶状体靶基因,影响 ARC 的病程。但出于伦理原因 Dismuke 等未检测正常人的房水外泌体,因此无法证明在 ARC 患者与正常人房水中外泌体 miRNA 的差异表达。Chen 等^[37]检测了合并高度近视与未合并高度近视的 ARC 患者房水中外泌体 miRNA 表达谱,但也不能反映 ARC 患者与正常人房水外泌体 miRNA 的表达差异。因此房水外泌体 miRNA 在 ARC 中的意义仍不清楚。

2.4 外泌体与青光眼 青光眼作为全球第二位致盲性眼病,病理性高眼压是其主要危险因素。根据前房角形态及病因可分为原发性闭角型青光眼(primary angle-closure glaucoma, PACG)和原发性开角型青光眼(primary open angle glaucoma, POAG)。POAG 与小梁网细胞外基质重塑导致的房水流出通道受阻有关^[38]。前文提到外泌体可携带 MMP^[22],提示外泌体可能参与 POAG 的细胞外基质重塑。Han 等^[39]报道,POAG 的发病与小梁网细胞的侵袭小体有关。侵袭小体是一种细胞膜伪足,表面含多种蛋白水解酶,参与降解细胞外基质。Hoshino 等^[40]报道,在侵袭小体形成过程中,特定的外泌体亚群被转运到侵袭小体内,当抑制外泌体相关基因后,可阻断侵袭小体形成及细胞外基质降解。Sung 等^[41]发现细胞迁移也依赖外泌体的释放。提示外泌体可能调控小梁网细胞的迁移、侵袭和细胞外基质重塑,但参与 POAG 发病的机制仍不明了。

MYOC 基因是 POAG 重要的致病基因,在小梁网及睫状肌中高表达^[42]。MYOC 基因编码 myocilin 蛋白,可调控小梁网细胞外基质沉积^[43]、小梁网细胞形态^[44]、睫状肌细胞外基质重塑^[45],是调节房水流出阻力的重要因子。房水中含 myocilin 蛋白,且主要存在于外泌体中^[46]。小梁网细胞与睫状肌细胞可通过房水外泌体实现信息交流^[47]。Stamer 等^[48]发现来源于房水和小梁网细胞株的外泌体中都有 myocilin 蛋白。地塞米松可诱导小梁网细胞株分泌的外泌体中 myocilin 蛋白显著增加。提示小梁网细胞外泌体 myocilin 在 POAG 和糖皮质激素相关性青光眼中具有潜在作用。

青光眼还与眼部炎症有关^[49]。人色素上皮细胞系 19 (adult retinal pigment epithelial cell line-19, ARPE-19)受白细胞介素 1B 刺激后,其分泌的外泌体可诱导单核细胞分泌多种炎症因子(如白细胞介素 6、白细胞介素 8、肿瘤坏死因子 α 等),并促进单核细胞凋亡^[16]。炎症因子诱导的 ARPE-19 外泌体亦可抑制 T 细胞增殖。提示 RPE 细胞外泌体可能参与减轻青光眼相关炎症反应。

青光眼病程中的高眼压会诱发视网膜神经节细胞(retinal ganglion cells, RGCs)凋亡,造成不可逆的视力损害。间充质干细胞外泌体不仅在角膜损伤中^[26],也在 RGCs 损伤中发挥保护作用。骨髓间充质干细胞分泌的外泌体可向受损的 RGCs 运输脑源性神经营养因子、神经生长因子和血小板源性生长因子,促进 RGCs 的存活和再生^[50]。间充质干细胞外泌体对 RGCs 的保护作用为治疗青光眼并发症提供了新思路。

2.5 外泌体与年龄相关性黄斑变性 ARMD 表现为 RPE、Bruch 膜和脉络膜毛细血管的退行性病变^[51]。局部慢性炎症和补体旁路途径失衡是 ARMD 的病理基础^[52]。当 RPE 细胞氧化应激时,外泌体可带走 RPE 细胞表面的免疫调节因子(如 CD46、CD55、CD59 等),使 RPE 细胞表面缺乏免疫调节因子而更易受到补体攻击^[53]。提示 RPE 外泌体可能参与 ARMD 相关的补体免疫。

Bruch 膜是脉络膜与 RPE 之间的屏障。ARMD 患者的 RPE 功能失调导致 Bruch 膜发生破坏,脉络膜毛细血管易穿透 Bruch 膜生长到视网膜形成 CNV,一旦累及黄斑区,会导致严重的视力损伤。Xu 等^[15]报道,人脐带胚胎干细胞分泌的外泌体可抑制缺氧 RPE 细胞的迁移与凋亡,为治疗 ARMD 提供了新思路。在 ARMD 早期,随着 RPE 的功能失调,黄斑区出现 Bruch 膜变薄和 RPE 下脂质与蛋白沉积,形成软性玻璃疣^[51]。在 ARMD 患者的视网膜玻璃疣中有外泌体标志物 CD63,且与玻璃疣相关淀粉样蛋白存在共定位^[54]。体外研究发现,RPE 细胞氧化损伤后溶酶体功能失调,胞吐作用增强^[54]。提示 RPE 细胞外泌体可能参与构成 ARMD 患者视网膜玻璃疣。此外,细胞外基质重塑与玻璃疣及新生血管密切相关^[55]。RPE 细胞会定向地从基底侧分泌含整合素样金属蛋白酶 10(a disintegrin and metalloprotease 10, ADAM10)的外泌体^[33]。ADAM 家族被发现参与细胞外基质重塑^[56]。提示 RPE 细胞外泌体可能参与调控 ARMD 中的细胞外基质重塑。

为探索外泌体对 ARMD 的诊断价值。Kang 等^[57]从 ARMD 患者的房水中提取到外泌体,发现与单纯 ARC 患者相比,ARMD 患者房水外泌体中组织蛋白酶 D (cathepsin D, CTSD)上调。接受抗 VEGF 单克隆抗体治疗后,患者房水外泌体中 CTSD 降低。在 ARPE-19 体外损伤模型的外泌体中也检测到 CTSD 上调。Voisin 等^[58]发现在 ARMD 患者和对照者(眼底检查正常,无视网膜玻璃疣或色素变性,且无 ARMD 家族史)提取的 RPE 多能干细胞中,ARMD 组的 CTSD 表达量更高。提示房水中的外泌体 CTSD 可作为判断 ARMD 进展的标志物。Klingeborn 等^[13]使用免疫亲和捕获法(以 CD81 作为抗原靶点)提取并比较了 ARPE-19 上清外泌体、人血浆外泌体、原代培养的猪 RPE 细胞顶端分泌的外泌体的蛋白表达谱,发现血浆外泌体与 ARPE-19 细胞外泌体蛋白表达谱有显著差异,且在血浆外泌体中未发现 RPE 细胞特异性的蛋白。由于尚未发现 ARMD 的血浆外泌体生物标志物,仍需寻找更具 RPE 细胞特异性的外泌体免疫捕获靶点。

2.6 外泌体与葡萄膜黑色素瘤 UM 是成年人最常见的眼内恶性肿瘤。外泌体可激活免疫系统调控 UM 的转移。非转移性 UM 可分泌外泌体至肿瘤细胞转移前微环境,递送 PEDF 激活单核细胞,并招募自然杀伤细胞(natural killer cell, NK 细胞)至肿瘤细胞转移前微环境。同时诱导巨噬细胞极化,并上调其肿瘤坏死因子相关凋亡诱导配体(TNF-related apoptosis-inducing ligand, TRAIL)的表达。NK 细胞和巨噬细胞均参与清除 UM 细胞,从而抑制非转移性 UM 的远处转移^[32]。而转移性 UM 分泌至肿瘤细胞转移前微环境的外泌体缺乏 PEDF,却携带有间质上皮转化因子^[59],能诱导血管渗漏和新生血管形成,促进 UM 转

移与生长。此外,Ragusa 等^[60]发现在 UM 患者玻璃体液中的外泌体 miR-146a 比对照者(正常健康眼球捐献者)上调,且 UM 患者的血清外泌体中 miR-146a 同样相对正常健康对照者升高。提示血清外泌体 miR-146a 有望成为 UM 诊断的潜在标志物。

3 总结和展望

作为一种普遍存在的跨细胞通讯方式,外泌体具有在细胞间传递生物活性分子调控靶细胞的功能。在免疫、退行性疾病、肿瘤、血管生成等各个领域,外泌体已成为研究热点。近年来,虽然在眼科疾病相关领域外泌体研究取得了一些进展,但仍相对处于起步阶段。外泌体研究技术的进步及眼科交叉领域的外泌体研究进展,势必会对眼科外泌体研究起到极大的推动作用。随着对外泌体在眼科疾病发生发展中作用的认识不断深入,外泌体有望成为眼病治疗的新兴靶点,也为眼病的诊断与预后提供了新思路。

参考文献

- 1 Rashed MH, Bayraktar E, Helal GK, et al. Exosomes: from garbage bins to promising therapeutic targets. *Int J Mol Sci* 2017; 18(3): 538
- 2 García-Seisdedos D, Babiy B, Lerma M, et al. Curcumin stimulates exosome/microvesicle release in an *in vitro* model of intracellular lipid accumulation by increasing ceramide synthesis. *Biochim Biophys Acta Mol Cell Biol Lipids* 2020; 1865(5): 158638
- 3 Messenger SW, Woo SS, Sun ZZ, et al. Correction: a Ca²⁺-stimulated exosome release pathway in cancer cells is regulated by Munc13-4. *J Cell Biol* 2019; 218(4): 1423
- 4 Yang L, Peng X, Li Y, et al. Long non-coding RNA HOTAIR promotes exosome secretion by regulating RAB35 and SNAP23 in hepatocellular carcinoma. *Mol Cancer* 2019; 18(1): 78
- 5 Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol* 2014; 30: 255-289
- 6 Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. *Cell Mol Life Sci* 2018; 75(2): 193-208
- 7 Lee YXF, Johansson H, Wood MJA, et al. Considerations and implications in the purification of extracellular vesicles-A cautionary tale. *Front Neurosci* 2019; 13: 1067
- 8 Colombo M, Moita C, van Niel G, et al. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. *J Cell Sci* 2013; 126(Pt 24): 5553-5565
- 9 Koh YQ, Almughliq FB, Vaswani K, et al. Exosome enrichment by ultracentrifugation and size exclusion chromatography. *Front Biosci (Landmark Ed)* 2018; 23: 865-874
- 10 Mori K, Hirase M, Morishige T, et al. A pretreatment-free, polymer-based platform prepared by molecular imprinting and post-imprinting modifications for sensing intact exosomes. *Angew Chem Int Ed Engl* 2019; 58(6): 1612-1615
- 11 Dismuke WM, Challa P, Navarro I, et al. Human aqueous humor exosomes. *Exp Eye Res* 2015; 132: 73-77
- 12 Katome T, Namekata K, Mitamura Y, et al. Expression of intraocular peroxisome proliferator-activated receptor gamma in patients with proliferative diabetic retinopathy. *J Diabetes Complications* 2015; 29(2): 275-281
- 13 Klingeborn M, Skiba NP, Stamer WD, et al. Isolation of retinal exosome biomarkers from blood by targeted immunocapture. *Adv Exp Med Biol* 2019; 1185: 21-25
- 14 Han KY, Chang JH, Azar DT. MMP14-containing exosomes cleave VEGFR1 and promote VEGFA-induced migration and proliferation of

- vascular endothelial cells. *Invest Ophthalmol Vis Sci* 2019; 60(6): 2321-2329
- 15 Xu ND, Huang LZ, Zhu L, et al. Human umbilical mesenchymal stem cells-derived exosomes modulate the proliferation, apoptosis and migration of human retinal pigment epithelial cells in hypoxia. *Zhonghua Yan Ke Za Zhi* 2019; 55(12): 933-941
- 16 Knickelbein JE, Liu B, Arakelyan A, et al. Modulation of immune responses by extracellular vesicles from retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 2016; 57(10): 4101-4107
- 17 Hajrasouliha AR, Jiang G, Lu Q, et al. Exosomes from retinal astrocytes contain antiangiogenic components that inhibit laser-induced choroidal neovascularization. *J Biol Chem* 2013; 288(39): 28058-28067
- 18 Bukowiecki A, Hos D, Cursiefen C, et al. Wound-healing studies in cornea and skin; parallels, differences and opportunities. *Int J Mol Sci* 2017; 18(6): 1257
- 19 Onguchi T, Han KY, Chang JH, et al. Membrane type-1 matrix metalloproteinase potentiates basic fibroblast growth factor-induced corneal neovascularization. *Am J Pathol* 2009; 174(4): 1564-1571
- 20 Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev* 2000; 14(17): 2123-2133
- 21 Meyer RD, Mohammadi M, Rahimi N. A single amino acid substitution in the activation loop defines the decoy characteristic of VEGFR-1/FLT-1. *J Biol Chem* 2006; 281(2): 867-875
- 22 Han KY, Dugas-Ford J, Seiki M, et al. Evidence for the involvement of MMP14 in MMP2 processing and recruitment in exosomes of corneal fibroblasts. *Invest Ophthalmol Vis Sci* 2015; 56(9): 5323-5329
- 23 Han KY, Tran JA, Chang JH, et al. Potential role of corneal epithelial cell-derived exosomes in corneal wound healing and neovascularization. *Sci Rep* 2017; 7: 40548
- 24 Zieske JD, Hutcheon AEK, Guo X. Extracellular vesicles and cell-cell communication in the cornea. *Anat Rec (Hoboken)* 2020; 303(6): 1727-1734
- 25 Shen T, Zheng QQ, Shen J, et al. Effects of adipose-derived mesenchymal stem cell exosomes on corneal stromal fibroblast viability and extracellular matrix synthesis. *Chin Med J (Engl)* 2018; 131(6): 704-712
- 26 Tao HY, Chen XN, Cao HM, et al. Mesenchymal stem cell-derived extracellular vesicles for corneal wound repair. *Stem Cells Int* 2019; 2019: 5738510
- 27 Shojaati G, Khandaker I, Funderburgh ML, et al. Mesenchymal stem cells reduce corneal fibrosis and inflammation via extracellular vesicle-mediated delivery of miRNA. *Stem Cells Transl Med* 2019; 8(11): 1192-1201
- 28 李雪, 张萍. 糖尿病视网膜病变的临床治疗新进展. *国际眼科杂志* 2019; 19(1): 69-72
- 29 Liu C, Ge HM, Liu BH, et al. Targeting pericyte-endothelial cell crosstalk by circular RNA-cPWWP2A inhibition aggravates diabetes-induced microvascular dysfunction. *Proc Natl Acad Sci U S A* 2019; 116(15): 7455-7464
- 30 Zhang W, Dong X, Wang T, et al. Exosomes derived from platelet-rich plasma mediate hyperglycemia-induced retinal endothelial injury via targeting the TLR4 signaling pathway. *Exp Eye Res* 2019; 189: 107813
- 31 Zhang W, Wang Y, Kong YC. Exosomes derived from mesenchymal stem cells modulate miR-126 to ameliorate hyperglycemia-induced retinal inflammation via targeting HMGB1. *Invest Ophthalmol Vis Sci* 2019; 60(1): 294-303
- 32 Plebanek MP, Angeloni NL, Vinokour E, et al. Pre-metastatic cancer exosomes induce immune surveillance by patrolling monocytes at the metastatic niche. *Nat Commun* 2017; 8(1): 1319

- 33 Klingeborn M, Dismuke WM, Skiba NP, *et al.* Directional exosome proteomes reflect polarity – specific functions in retinal pigmented epithelium monolayers. *Sci Rep* 2017; 7(1): 4901
- 34 Wu CR, Lin HT, Wang QL, *et al.* Discrepant expression of microRNAs in transparent and cataractous human lenses. *Invest Ophthalmol Vis Sci* 2012; 53(7): 3906–3912
- 35 Wu CR, Ye M, Qin L, *et al.* Expression of lens-related microRNAs in transparent infant lenses and congenital cataract. *Int J Ophthalmol* 2017; 10(3): 361–365
- 36 Wu CR, Liu Z, Ma L, *et al.* MiRNAs regulate oxidative stress related genes via binding to the 3' UTR and TATA – box regions; a new hypothesis for cataract pathogenesis. *BMC Ophthalmol* 2017; 17(1): 142
- 37 Chen CF, Hua KT, Woung LC, *et al.* Expression profiling of exosomal miRNAs derived from the aqueous humor of myopia patients. *Tohoku J Exp Med* 2019; 249(3): 213–221
- 38 Roy Chowdhury U, Hann CR, Stamer WD, *et al.* Aqueous humor outflow; dynamics and disease. *Invest Ophthalmol Vis Sci* 2015; 56(5): 2993–3003
- 39 Han H, Kampik D, Grehn F, *et al.* TGF- β 2-induced invadosomes in human trabecular meshwork cells. *PLoS One* 2013; 8(8): e70595
- 40 Hoshino D, Kirkbride KC, Costello K, *et al.* Exosome secretion is enhanced by invadopodia and drives invasive behavior. *Cell Rep* 2013; 5(5): 1159–1168
- 41 Sung BH, Ketova T, Hoshino D, *et al.* Directional cell movement through tissues is controlled by exosome secretion. *Nat Commun* 2015; 6: 7164
- 42 van der Heide CJ, Alward WLM, Flamme – Wiese M, *et al.* Histochemical analysis of *Glaucoma* caused by a myocilin mutation in a human donor eye. *Ophthalmol Glaucoma* 2018; 1(2): 132–138
- 43 Fautsch MP, Bahler CK, Jewison DJ, *et al.* Recombinant TIGR/MYOC increases outflow resistance in the human anterior segment. *Invest Ophthalmol Vis Sci* 2000; 41(13): 4163–4168
- 44 Joe MK, Sohn S, Hur W, *et al.* Accumulation of mutant myocilins in ER leads to ER stress and potential cytotoxicity in human trabecular meshwork cells. *Biochem Biophys Res Commun* 2003; 312(3): 592–600
- 45 Lindsey JD, Gatton DD, Sagara T, *et al.* Reduced TIGR/myocilin protein in the monkey ciliary muscle after topical prostaglandin F (2 α) treatment. *Invest Ophthalmol Vis Sci* 2001; 42(8): 1781–1786
- 46 Perkumas KM, Hoffman EA, McKay BS, *et al.* Myocilin-associated exosomes in human ocular samples. *Exp Eye Res* 2007; 84(1): 209–212
- 47 Hoffman EA, Perkumas KM, Highstrom LM, *et al.* Regulation of myocilin-associated exosome release from human trabecular meshwork cells. *Invest Ophthalmol Vis Sci* 2009; 50(3): 1313–1318
- 48 Stamer WD, Hoffman EA, Luther JM, *et al.* Protein profile of exosomes from trabecular meshwork cells. *J Proteomics* 2011; 74(6): 796–804
- 49 Kalogeropoulos D, Sung VC. Pathogenesis of uveitic Glaucoma. *J Curr Glaucoma Pract* 2018; 12(3): 125–138
- 50 Harrell CR, Fellabaum C, Arsenijevic A, *et al.* Therapeutic potential of mesenchymal stem cells and their secretome in the treatment of Glaucoma. *Stem Cells Int* 2019; 2019: 7869130
- 51 Blasiak J. Senescence in the pathogenesis of age – related macular degeneration. *Cell Mol Life Sci* 2020[Epub ahead of print]
- 52 Yaspan BL, Williams DF, Holz FG, *et al.* Targeting factor D of the alternative complement pathway reduces geographic atrophy progression secondary to age-related macular degeneration. *Sci Transl Med* 2017; 9(395): eaaf1443
- 53 Ebrahimi KB, Fijalkowski N, Cano M, *et al.* Oxidized low-density-lipoprotein-induced injury in retinal pigment epithelium alters expression of the membrane complement regulatory factors CD46 and CD59 through exosomal and apoptotic bleb release. *Adv Exp Med Biol* 2014; 801: 259–265
- 54 Wang AL, Lukas TJ, Yuan M, *et al.* Autophagy and exosomes in the aged retinal pigment epithelium; possible relevance to drusen formation and age-related macular degeneration. *PLoS One* 2009; 4(1): e4160
- 55 Nita M, Strzałka – Mrozik B, Grzybowski A, *et al.* Age – related macular degeneration and changes in the extracellular matrix. *Med Sci Monit* 2014; 20: 1003–1016
- 56 White JM. ADAMs: modulators of cell – cell and cell – matrix interactions. *Curr Opin Cell Biol* 2003; 15(5): 598–606
- 57 Kang GY, Bang JY, Choi AJ, *et al.* Exosomal proteins in the aqueous humor as novel biomarkers in patients with neovascular age – related macular degeneration. *J Proteome Res* 2014; 13(2): 581–595
- 58 Voisin A, Monville C, Plancheron A, *et al.* Cathepsin B pH – dependent activity is involved in lysosomal dysregulation in atrophic age-related macular degeneration. *Oxid Med Cell Longev* 2019; 2019: 5637075
- 59 Peinado H, Alečković M, Lavotshkin S, *et al.* Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med* 2012; 18(6): 883–891
- 60 Ragusa M, Barbagallo C, Statello L, *et al.* miRNA profiling in vitreous humor, vitreal exosomes and serum from uveal melanoma patients: Pathological and diagnostic implications. *Cancer Biol Ther* 2015; 16(9): 1387–1396