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REVIEW
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Therapeutic Exosomes in Prognosis and Developments of Coronary Artery Disease

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Exosomes, with an diameter of 30∼150 nm, could be released from almost all types of cells, which contain diverse effective constituent, such as RNAs, proteins, lipids, and so on. In recent years, exosomes have been verified to play an important role in mechanism, diagnosis, treatment, and prognosis of cardiovascular disease, especially coronary artery disease (CAD). Moreover, it has also been shown that exosomes derived from different cell types have various biological functions based on the cell stimulation and microenvironment. However, therapeutic exosomes are currently far away from clinical translation, despite it is full of hope. In this review, we summarize an update of the recent studies and systematic knowledge of therapeutic exosomes in atherosclerosis, myocardial infarction, and in-stent restenosis, which might provide a novel insight into the treatment of CAD and promote the potential clinical application of therapeutic exosomes.

Keywords: exosomes, CAD, atherosclerosis, myocardial infarction, drug delivery

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INTRODUCTION

Coronary artery disease (CAD) still remains a high-prevalence, high-risk, and high-fatality cardiovascular disease worldwide. In spite of the profound development of device and agents in CAD treatment, the prognosis of CAD, especially acute myocardial infarction, is far from being satisfactory (1, 2). Recently, exosome emerges as a novel, full of hope, and potential alternative to cell-based therapies of CAD due to its cardioprotective properties (3).

Exosomes, with diameter of 30∼150 nm and density of 1.13∼1.19 g/ml, are the smallest extracellular vesicles (EVs) (4), with a bilayer membrane structure released by almost all types of cells (5, 6). The biogenesis of exosomes triggers from membrane proteins being endocytosed via inward budding of the cell membrane, which are then transferred to early endosomes (EEs). Afterwards, the EEs mature into multivesicle bodies (MVBs), filled with numerous intraluminal vesicles (ILVs) (7, 8), which incorporate proteins, lipids, and genetic material during invagination (9). Finally, MVBs can fuse with cell membrane and release ILVs to the extracellular space (10), as we call them exosomes, or result in degradation via fusing with lysosomes (**Figure 1**) (11).

However, therapeutic exosomes are currently far away from clinical application, in spite of so many outstanding qualities of exosomes. In this review, we will summarize an update of the recent findings and systematic knowledge of therapeutic exosomes in CAD, which might provide a novel insight into the treatment of CAD and promote the potential clinical translation of therapeutic exosomes.

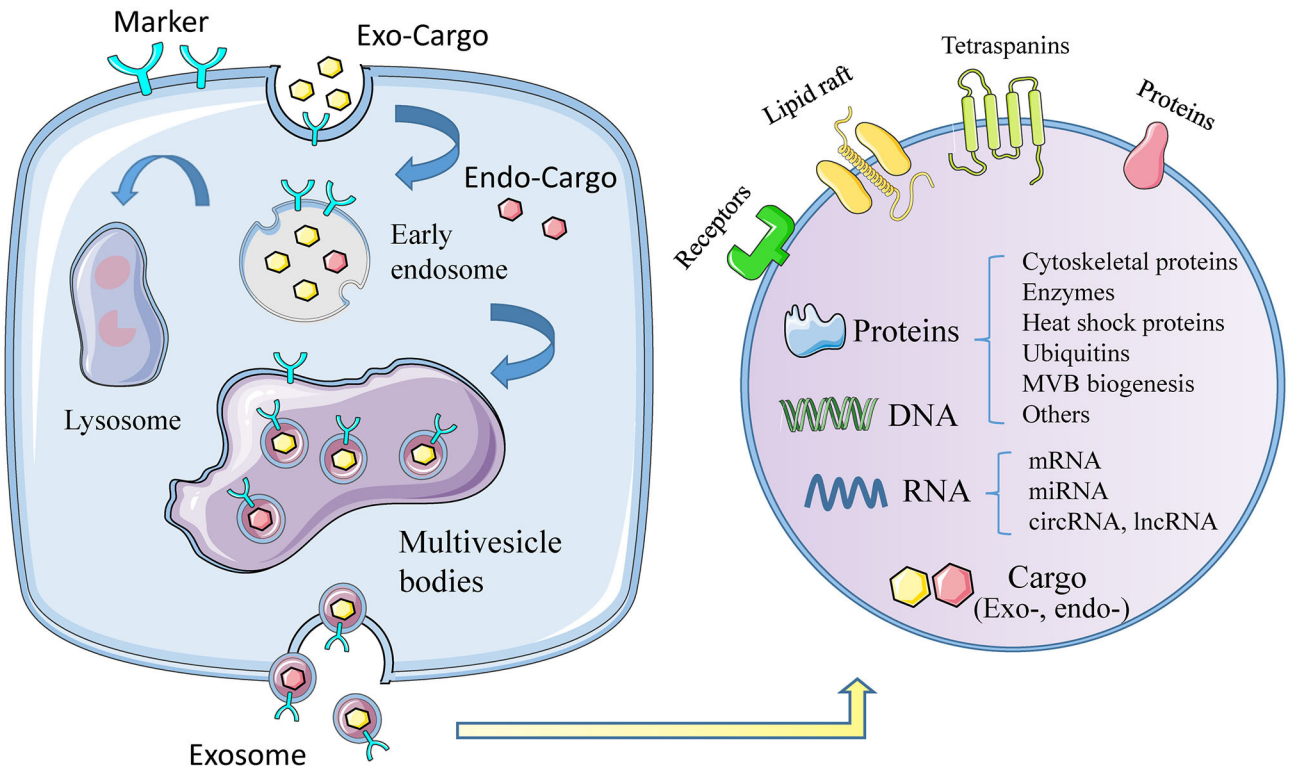

FIGURE 1 | The biogenesis, formation, and content of exosomes. The formation of exosomes begins with invagination of the plasma membrane, and then forms early exosomes, which form multiple vesicles after fusion, and finally release the exosomes out of the cell. During the formation of exosomes, it will actively or passively carry exogenous or endogenous cargoes. The picture on the right shows the membrane structure of exosomes, the contents of exosomes, including proteins, DNA, RNA, and others.

EXOSOMES AND CAD

According to the progress of CAD, the relationships between exosomes and CAD are summarized into three parts: exosomes in the prevention of atherosclerosis, exosomes in the diagnosis and treatment of myocardial infarction, and exosomes in the development of in-stent restenosis (**Table 1**).

Therapeutic Exosomes in Atherosclerosis

A basic progress in the development of atherosclerosis is monocytes/macrophages accumulation into the vessel wall to produce pro-inflammatory cytokines (32). It has been reported that molecularly engineered M2 macrophage-derived exosomes (Further electroporated with hexyl 5-aminolevulinate hydrochloride) alleviated inflammation by promoting the release of anti-inflammatory cytokines (33). Paeonol could restrict atherosclerosis by obviously increasing miR-223 expression in exosomes from monocytes and inhibiting STAT3 pathway (34). Exosomes laden with heat shock protein 27 (HSP27) significantly stimulated NF-κB activation and IL-10 release, suggesting that exosomes could act as a vector in anti-inflammatory therapy (35). Mitochondria constituted a major subset of extracellular vesicles released by LPS-activated monocytes *in vitro*, which were associated with type I IFN and TNF signaling (36). Exosomes from nicotine-stimulated macrophages could promote atherosclerosis through facilitating VSMC migration and proliferation by targeting miR-21-3p/*PTEN* (37). Moreover, helicobacter pylori-infected gastric epithelial cells-derived exosomes accelerated macrophage foam cell formation and promoted atherosclerosis by CagA (38). Insulin resistance adipocyte-derived exosomes (IRADEs) has been reported to aggravate the plaque burden, whereas its effect could be attenuated by silencing sonic hedgehog in IRADEs (12). Besides, Jiang et al. (13) also reported that steatotic hepatocyte-derived EVs promoted endothelial inflammation by miR-1 delivery, KLF4 suppression and the NF-κB pathway activation. And in this instance, exosome therapy might be the reduction of negative contents in exosomes such as miR-1 instead of increasing therapeutic exosomes.

Therapeutic Exosomes in Myocardial Infarction

Myocardial infarction, which often results in poor clinical outcomes, still remains the lack of effective treatment, especially for those without culprit vessel revascularization (14). Therefore, current clinical treatments are mostly based on easinesss of symptoms rather than repairing infarcted cardiomyocyte (15).

TABLE 1 | Relationship between exosomes and CAD.

Disease	Exosomal cargo	Parent cells	Recipient cells	Target	Biological/clinical relevance	Reference
AS	miR-223	THP-1 monocyte	HUVEC	STAT-3 pathway	Anti-inflammation	(12)
	HSP27	THP-1 monocyte	–	NF-κB, IL-10	Anti-inflammation	(13)
	Mitochondria	Monocyte	Endothelial cell	IFN, TNF	Anti-inflammation	(14)
	miR-1	Hepatocyte	Endothelial cell	KLF4, NF-κB	Anti-inflammation	(15)
	miR-21-3p	MACROPHAGE	VSMC	PTEN	Promote VSMC proliferation and degradation	(16)
	–	Gastric epithelial cell	Macrophage	CagA	Promote foam cell formation	(17)
	Sonic hedgehog	Adipocyte	HUVECs, MAECs	TGF-α, IL-1β, IL-6	Reduce plaque vulnerability	(18)
MI	miR-342-5p	Endothelials	CMs	Caspase9, Jnk2, Akt	Anti-apoptsis/proliferation	(19)
	miR-21	HEK293T cell	CMs, HUVECs	PDCD4	Anti-apoptosis	(20)
	miR-125b-5p	MSC	CMs	p53, BAK1	Anti-apoptosis	(21)
	miR-210	EPC	Endothelial cell	Mitochondria	Anti-apoptosis/promote angiogenic function	(22)
	miR-24	Serum	H9c2 cell	Bim	Mediate Remote ischemic preconditioning	(23)
	miR-93-5p	Adipose stromal cell	CMs	Atg7, TLR4	Inhibit autophagy, anti-inflammatory	(24)
	lncR	–	Fibroblast, CMs	Neat1	Anti-fibrosis	(25)
	miR-24	MSC	CD8+T	Bim	Anti-fibrosis	(26)
	miR-130-3p	Adipocyte	CMs	AMPKα1/α2, Birc6, and Ucp3	Anti-apoptosis (diabetic)	
	Cytotoxic substance	Serum	HL-1 CMs	Compliment C4, ApoE, Apo C-IV	Anti-apoptosis (diabetic)	(27)
	ILK	Progenitor	CMs	NF-κB	Enhance myocardial repair	(28)
ISR	miR-222	M1-macrophages	VSMC	CDKN1B/CDKN1C	Promote VSMC proliferation and degradation	(29)
	miR-125b	MSC	VSMC	Myosin-1E	Promote VSMC proliferation and degradation	(30)
	miR-21-5p	EPC	HUVEC	THBS1	Promote repair of endothelial cells	(31)

AS, atherosclerosis; MI, myocardial infraction; ISR, in stent restenosis; MSC, mesenchymal stem cell; EPC, endothelial progenitor cell; CM, cardiomyocyte; VSMC, vascular smooth muscle cell; HUVEC, human umbilical vein endothelial cell.

Exosomes reveal significant anti-apoptosis of cardiomyocyte after myocardial infarction. Exercise-derived exosomal miR-342-5p inhibited cardiomyocyte apoptosis by targeting *Caspase9* and *Jnk2* after left anterior descending artery occlusion (16). EVs overexpressing miR-21 could dramatically reduce PDCD4 expression and alleviate myocardial apoptosis (15). Hypoxia-conditioned bone marrow-mesenchymal stem cells (MSCs)-derived exosomes (Hypo-Exo) could also protect cardiomyocytes from apoptosis by enrichment of miR-125b-5p and suppressing the expression of genes *p53* and *BAK1* (17). In addition, miR-210 in endothelial progenitor cell-derived exosomes (EPC-EXs) possessed antiapoptotic functions onto hypoxia/reoxygenation-injured human endothelial cells (18). Remote ischemic preconditioning-induced exosomes (RIPC-Exo) also could transfer miR-24 into myocardium to inhibit apoptosis (39).

Exosomes also provide cardioprotection by activating cell survival signals, inhibiting inflammatory factors, delaying ventricular remodeling, and reducing myocardial fibrosis after the occurrence of myocardial infarction. Exercise-derived exosome (Ex-exo) could carry miR-342-5p to promote Akt phosphorylation by targeting gene *Ppmlf* (16). MiR-93-5p in adipose stromal cell-derived exosomes (ADSC-Exo) inhibited inflammatory response and prevented myocardial infarction by targeting *Atg7* and *TLR4* (20). Kenneweg et al. (19) had reported that fibroblasts absorbed lncR-EVs and promoted myocardial fibrosis by targeting *Neat1*. Moreover, exosomal miR-24, derived from allogenic human umbilical MSC, could inhibit cardiac fibrosis (21).

Patients suffering from myocardial infarction often have a history of diabetes. Gan et al. (22) had demonstrated that the enrichment of miR-130b-3p from dysfunctional adipocyte exacerbated myocardial infarction and cardiomyocyte apoptosis. Serum-exosomes from normoglycemic rats could alleviate the death of hypoxia/reoxygenation-induced *HL-1* cell, however, which disappears in type-2 diabetes rat model (23).

Exosomes also can serve as an adjuvant therapy. Integrin Linked Kinase (ILK) acted as a target kinase by which progenitor cell-derived exosomes attenuated myocardial injury (24). Cheng et al. (25) have reported that miRNA in EVs contributed to early detection of CAD by means of point-of care applications.

Therapeutic Exosomes in In-stent Restenosis

Percutaneous coronary intervention has become a very important treatment strategy for CAD, but in-stent restenosis is blamed for the main cause of stent failure in patients with CAD (26, 40). Several previous studies have shown that the risk of in-stent restenosis in CAD patients undergoing coronary stent implantation during 1 year follow-up was ∼5–10% (27). The underlying mechanisms of in-stent restenosis are quite complex, and at least exosomes play a crucial role in the development of in-stent restenosis. For example, miR-222 from M1 macrophages (M1M)-derived exosomes promoted vascular smooth muscle

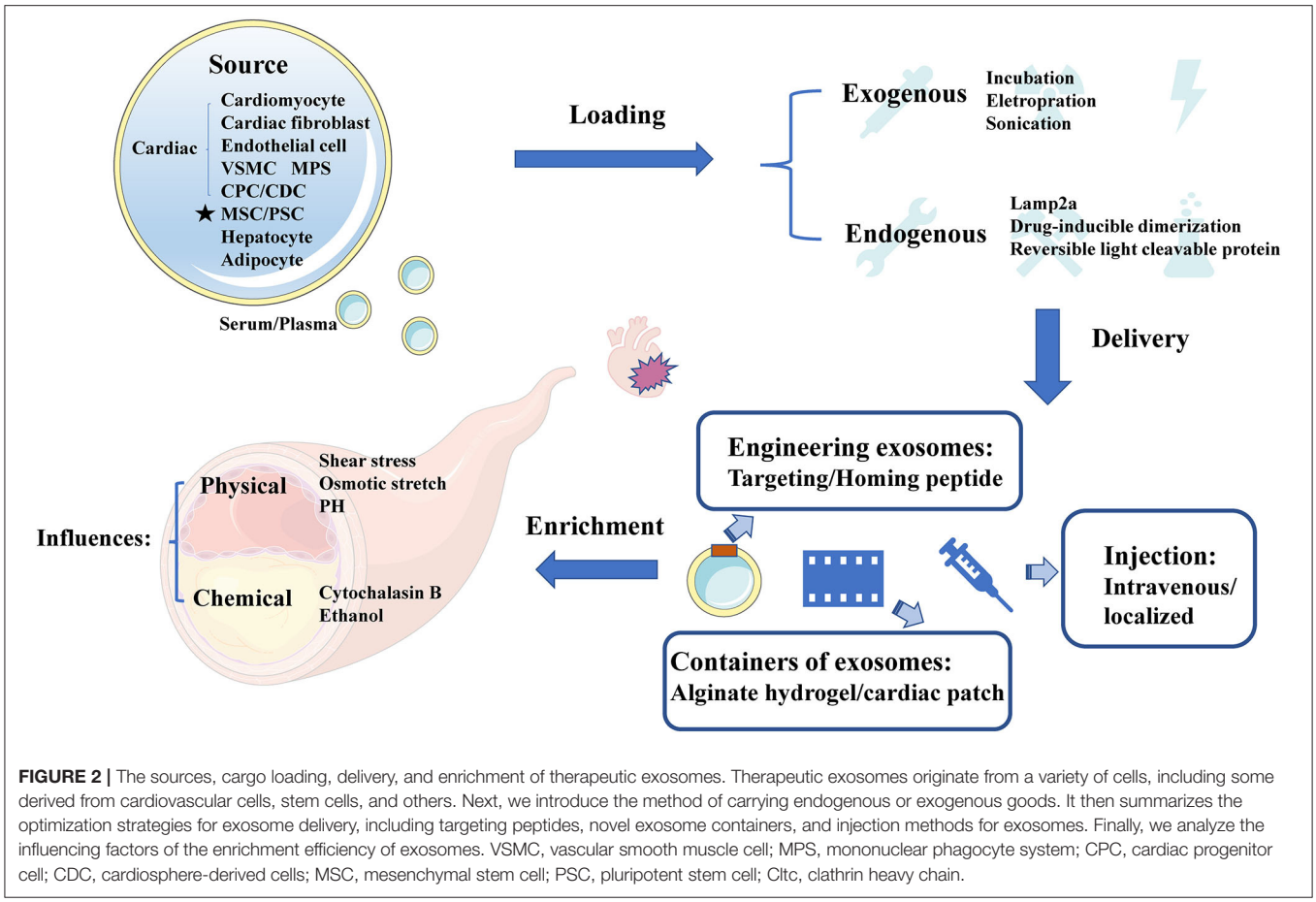

FIGURE 2 | The sources, cargo loading, delivery, and enrichment of therapeutic exosomes. Therapeutic exosomes originate from a variety of cells, including some derived from cardiovascular cells, stem cells, and others. Next, we introduce the method of carrying endogenous or exogenous goods. It then summarizes the optimization strategies for exosome delivery, including targeting peptides, novel exosome containers, and injection methods for exosomes. Finally, we analyze the influencing factors of the enrichment efficiency of exosomes. VSMC, vascular smooth muscle cell; MPS, mononuclear phagocyte system; CPC, cardiac progenitor cell; CDC, cardiosphere-derived cells; MSC, mesenchymal stem cell; PSC, pluripotent stem cell; Cltc, clathrin heavy chain.

cells (VSMCs) proliferation and migration, which resulted in restenosis (41). Wang et al. (42) reported that MSC-Exo enriched miR-125b and inhibited the proliferation and migration of VSMC by targeting myosin 1E. Moreover, EPC-Exo also were involved in the prevention of restenosis through delivering miR-21-5p and inhibiting *THBS1* expression (43). Recently, exosome-eluting stents have been proven to reduce intimal hyperplasia and accelerate re-endothelialization in the ischemic injury rat model.

OPTIMIZED TREATMENT STRATEGY

Exosomes appear superiority and irreplaceable biological functions, and the clinical application of therapeutic exosomes is full of hope. In the first place, exosomes can avoid phagocytosis and bypass the engulfment by lysosomes (44) to exhibit a longer circulation half-life due to the protection of phospholipid bilayer membrane (28). Secondly, phospholipid bilayer of exosomes is also beneficial to the fusion with membrane of recipient cells (29). Thirdly, exosomes derived from animals or patients have the high homolog and low immune response to avoid exosomes degradation (30). Finally, exosomal regulation of "Homing" effect has been reported to target the cell type where exosomes were produced (31), which can provide a shortcut for exosomes delivery. In need of optimized treatment strategy, we summarized the latest research involved of sources, cargo loading, delivery and enrichment of therapeutic exosomes (**Figure 2**).

Source of Therapeutic Exosomes

It has been reported that the sources of CAD related therapeutic exosomes were commonly cardiovascular-derived endothelial cells, smooth muscle cells, macrophages and cardiac fibroblasts (45). In recent years, several studies have highlighted the value of MSC-Exo therapy in cardiac protection (46, 47), and MSC could secret the highest amount of exosomes (48). Moreover, other studies found that circulating-Exo, adipocyte-EVs (12), hepatocyte-EVs (13), accompanied with different degrees of heterogeneity, all existed therapeutically effect upon CAD.

Loading Therapeutic Cargo in Exosomes

Although many therapeutic cargoes are inherent in parent cells previously, some therapeutic cargoes could only be loaded into exosomes by artificial means. Normally, cargoes could be loaded through fusion with liposomes, adsorption of molecules to the surface of exosomes and the insertion of lipids (49). It has been reported that a few procedures, such as incubation, electroporation (33), sonication (50), and so on (51), could promote cargo loading. When choosing the loading method of

cargoes, we should consider the loading efficiency (52), and whether this loading method will change the physical and chemical characteristics of exosomes (53). Besides, membrane protein Lamp2a could increase the loading of miRNA into EVs (54). Moreover, drug-inducible dimerization (55), reversible light cleavable protein (56), and several advanced means of engineering exosomes also contribute to the loading of endogenous cargoes.

Delivery Method

Normally, therapeutic exosomes were injected intravenously and act on the cardiovascular diseases through the circulatory system as an essential treatment. However, most of these exosomes are taken up by liver or spleen (57). Loading homing peptides has become a popular way to optimize delivery of exosomes (58). In cardiovascular field, several homing peptides in connection with atherosclerosis (59, 60), and ischemia/reperfusion–injured cardiomyocytes (61) have been identified and applied in therapeutic regimen. For example, Wang et al. (62) have demonstrated that engineered exosomes fused with ischemic myocardium-targeting peptide (IMTP) increasingly accumulated in ischemic heart area. Furthermore, it has been reported that exosomes conjugated with cardiac homing peptide (CHP) has higher retention in infarcted heart (63).

Besides, Song et al. (15) have reported that localized injection of EVs attenuated the apoptosis of cardiomyocytes and endothelial cells in a preclinical myocardial infarction (MI) animal model. To reduce losses during transportation, Lv et al. (64) have reported that sEVs, incorporated in alginate hydrogel, act as a new regimen of therapy. An off-the-shelf therapeutic cardiac patch, composed of extracellular matrix and cardiac stromal cells (CSC), has been confirmed in the model of MI (65). The examples above demonstrate the superiority of local delivery of exosomes and improve the retention rate of exosomes.

Enrichment Efficiency

The enrichment efficiency of exosomes is affected by physical and chemical stimuli. The physical stimulation of exosomes mainly includes shear stress, osmotic stretch, PH and others (66). More importantly, the change of blood flow shear force, as the initiating factor of coronary artery disease, has also become a difficult problem for exosome delivery. Here, we focus on the shear stress in vessel where exosomes were regulated. While shear stress remain within 1–70 dynes/cm^2 in normal blood vessels, severely narrowed blood vessels can produce over 1,000 dynes/cm^2 (67). High shear stress, occurring in atherosclerotic arteries, could accelerate the release of circulating-EVs gradually (68). The mechanisms of shear stress on EVs secretion relate to the response of membrane tension (69). Besides, calcium could enhance exosomes secretion from a microenvironment perspective (70), whereas arterial hypertension was also associated with the increase of shear stress from a macro perspective (71). Evidence proved that exercise training could increase EVs release under high shear stress, and decrease the risk of thrombosis correspond to stenotic arteries (72). Exosomes could also be affected by chemical trigger, including cytochalasin B and ethanol (46).

CONCLUSION AND FUTURE PERSPECTIVE

In recent years, the therapeutic effect of exosomes on heart diseases has been gradually discovered. We have summarized the progress in studying exosomes as drug delivery vehicles. Before entering the clinical transformation, a perfect therapeutic concept of exosomes is essential (3), and pioneering in the field of exosomes is tumor-related studies. We can draw on tumor-related studies to optimize treatment regimens. Certainly, CAD-targeted treatment options also need to take notice of the cardiovascular lineage specificity.

Exosomes, as natural drug delivery vehicles, have excellent biocompatibility and targeting properties. We have discovered the potential of exosomes in the treatment of CAD based on existing research. However, exosomes still face huge resistance in clinical transformation. Moreover, we hope that the optimization of therapeutic exosomes is getting better and enter the clinical application stage as soon as possible.

AUTHOR CONTRIBUTIONS

A-QC and X-FG wrote the manuscript. Z-MW and FW prepared the figures. SL and YG prepared the table. J-JZ and S-LC provided the idea and revised the manuscript. All authors have agreed to the published version of the manuscript.

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