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NEONATAL ISLET-1+ CARDIOVASCULAR PROGENITOR CELL-DERIVED EXOSOMES AND THEIR FUNCTIONAL ABILITY TO REGULATE PROLIFERATION

Lourdes Ceja

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A Thesis

Presented to the

Faculty of

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In Partial Fulfillment

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in

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by

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ABSTRACT

Heart failure affects many people around the world and can lead to disease progression and death. Consequently, new stem and exosome-based therapies are needed to address this major health issue and to provide therapeutic options that will improve outcomes for the increasing number of patients with heart disease. Stem cell-derived exosomes have captivated researchers' attention over the past couple of years based on their functional role in cellular signaling which highlights the vital component of the secretome of stem and progenitor cells. Since neonates have significantly enhanced regenerative ability, we hypothesized that exosomes isolated from Islet-1+ expressing neonatal human cardiovascular progenitors (CPCs) will induce transcriptomic changes associated with improved regenerative capability when co-cultured with CPCs derived from adult humans. In order to test this hypothesis, we isolated exosomes from human neonatal Islet-1+ cardiovascular progenitor cell clones, analyzed the exosome content using RNAseq and treated adult CPCs with exosomes derived from neonatal CPCs to assess the functional effect. Analysis of neonatal CPC-derived exosome content by RNAseq revealed that neonatal exosomes contain miRNAs that inhibit YAP1 repressors and expressed transcripts predicted to activate YAP1, the cell cycle, and GPCR signaling. In vitro, adult CPCs treated with exosomes derived from neonatal CPCs demonstrated activation of AKT signaling, which promotes survival and proliferation and several transcripts involved in proliferation and cell cycle

progression, including YAP1. YAP1 is lost after the neonatal period under normal development but can stimulate cardiac regeneration. Our results demonstrate that transcripts associated with enhanced regenerative effects can be induced in adult CPCs following treatment with neonatal CPC-derived exosomes. Our data suggests that neonatal Islet-1+ CPC exosome content can provide a stimulus that may improve functional outcomes when adult CPCs are used for cell-based cardiovascular repair.

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CHAPTER ONE

INTRODUCTION

Background

Current Limitations in Heart Repair

Heart failure is a major cause of high morbidity and mortality rates (Camps-Vilaró et al., 2020; Dagenais et al., 2020). From a global perspective, heart failure affects 64 million people (Savarese et al., 2022). Heart failure is a syndrome which presents from any structural or functional cardiac abnormality and impairs ventricular filling or ejection of blood (Yancy et al., 2013). In the adult heart, a minimal potential for regenerative capacity exists and the loss of cardiomyocytes that occurs through myocardial injury such as myocardial infarction, is irreversible (Weinberger & Eschenhagen, 2021). After myocardial infarction, cardiac remodeling occurs (Garza et al., 2015). Remodeling includes hypertrophy; an enlargement of the size of cardiomyocytes and thickening of ventricular walls following proliferation of fibroblasts and accumulation of extracellular matrix proteins. This process affects cardiomyocyte biomechanical signaling and ultimately function (Münch & Abdelilah-Seyfried, 2021; Sutton & Sharpe, 2000). As a result, myocardial injury leads to a decline in left ventricular function and immediate recovery is both rare and incomplete (Weinberger &

Eschenhagen, 2021). This is because cardiomyocytes exit the cellular cycle after birth and do not proliferate to successively replace the lost tissue (Ahuja et al., 2007; Powers & Huang, 2022). The rate of modest cardiomyocyte renewal in the human adult is about 1% turnover per year and declines to approximately 0.45% by the age of 75 (Bergmann et al., 2009, 2015). Rare proliferative events can occur within the adult myocardium, but terminally differentiated cardiomyocytes in the adult human heart would need to successfully enter the cellular cycle in sufficient numbers in order to induce cardiovascular repair (Günthel et al., 2018). Regimens which are currently used for the treatment of myocardial infarction consequently focus on targeting the resident cardiomyocyte neurohormonal physiology rather than attempting to replenish the missing cardiomyocytes (Sharma et al., 2015). Alternative approaches aimed at promoting proliferation in resident cardiac stem cells to stimulate cardiovascular regeneration after myocardial infarction would be beneficial.

Stem Cells for Heart Repair

Stem cells such as human embryonic stem cells, induced pluripotent stem cells (iPSCs), Islet-1+ expressing progenitors, and cardiac progenitor cells (CPCs) are being examined as potential sources of cells that can repair damaged tissue (Bartulos et al., n.d.; Ghazizadeh et al., 2018; reviewed in Kasai-Brunswick et al., 2021). Embryonic stem cells are equipped with pluripotency and

can be differentiated into functional cardiomyocytes (Kehat et al., 2001; Yamanaka et al., 2008). Molecular and functional properties of embryonic stem cells and IPSCs share similarity and in the case of iPSCs, the use of stem cell derivatives that are matched to the recipient eliminates the potential for rejection of cells that were introduced (Choi et al., 2015; Ortuño-Costela et al., 2019). The use of iPSCs from a histocompatible source different from the potential recipient is also possible, however these cells may contain genetic variations based on the source cell population or alterations that occur due to reprogramming and maintenance. These variations could be problematic (Deleidi et al., 2011; G. Liang & Zhang, 2013). Additionally, if a population of differentiated human embryonic stem cells or iPSCs remained undifferentiated, a possibility of teratoma formation exists *in vivo* (Hentze et al., 2009; A. S. Lee et al., 2009; M.-O. Lee et al., 2013). Using human embryonic stem cells raises ethical and safety concerns, because of their derivation and their tumorigenic potential (Deinsberger et al., 2020; Q. Sun et al., 2014). iPSC's also raise ethical and safety concerns based on the potential for undesired differentiation and tumorigenic transformation as well as issues regarding donor's privacy and intellectual property (Moradi et al., 2019; Volarevic et al., 2018). These concerns can be a limitation for future clinical applications.

Cardiovascular Progenitor Cells

Another approach would be the use of cardiac progenitor cells, which have shown promise as a therapeutic model (reviewed in Bollini et al., 2011; reviewed in Witman et al., 2020a). Cardiac progenitors are a multipotent self-renewing progenitor cell type arising from a subpopulation of mesodermal lineage precursors found at early cardiac stages of embryogenesis (Devine et al., n.d.; Mauretti et al., 2017; Witman et al., 2020b; S. M. Wu et al., 2006). Over the past few years, preclinical and clinical studies have utilized various sources of endogenous stem cells for the treatment of several cardiovascular diseases such as heart failure and myocardial infarction (reviewed in Fan et al., 2021; reviewed in Hou & Li, 2018). There are various cardiac progenitor cell populations identified that provide a better cell source for cardiovascular therapy (Le & Chong, 2016). To date, the results in patients with acute myocardial infarction and the use of cardiac stem cell-derived therapy using c-kit+ cells has only shown moderate improvement in therapeutic outcomes (Kasai-Brunswick et al., 2021b). Therefore, a substantial clinical need for an optimal stem-cell therapy to address heart failure remains.

Islet-1+ Expressing Progenitors as a Source for Cardiac Repair

Islet-1+ expressing cardiac progenitor cells isolated from patients possess a unique differentiation potential and represent the cell population studied in our

laboratory (Fuentes et al., 2013a). Cells which are Islet-1+ have been shown to be essential in the cardiac developmental process based on a study using Islet-1 deficient mice which lacked all structures derived from the second heart field (Cai et al., 2003). In another study, they observed that deletion of Islet-1 in mice leads to early embryonic lethality (Gao et al., 2019). Islet-1 expression in the early stages of cardiac development has been identified as a marker of cells of the second heart field, cardiac neural crest, and the proepicardium when co-expressed with Nkx2.5 (Le & Chong, 2016; Shouman et al., 2021). Islet-1+ cardiovascular progenitors have shown promise in cell-based cardiovascular repair models (Bartulos et al., 2016; Foo et al., 2018; Y. Li et al., 2017). In an *in vivo* study using murine Islet-1+ CPC spheroids, there was significant improvement in left ventricular function and reduction in scar size in treated mice four weeks after injury (Bartulos et al., 2016). In an *in vivo* myocardial infarction model, Islet-1+ human embryonic stem cell-derived ventricular progenitors were injected into the myocardium of mice and resulted in preservation of myocardial contractile function (Foo et al., 2018). Islet-1+ cells represent a population of unique, rare, self-renewable, multipotent cells and are able to differentiate into the three cardiac lineages; cardiomyocytes, smooth muscle cells, and endothelial cells (Laugwitz et al., 2008; Y. Li et al., 2017). Our laboratory has established Islet-1+ neonatal and adult cardiac progenitor cell clones by single cell expansion to serve as a model for cardiovascular repair (Baio et al., 2018; Fuentes et al.,

2013a). The use of single cell clones with a well-defined phenotype optimizes the safety profile and reproducibility for studies focused on the application of either stem cells or stem cell-derived exosomes as a model for cell-based cardiovascular repair.

Stem and Progenitor Cell-derived Exosomes

Stem cell-derived exosomes have served as a vital component of the secretome of stem and progenitor cells, based on their functional role in cellular signaling over the past couple of years (reviewed in Balbi & Vassalli, 2020; reviewed in Jayaraman et al., 2021). Recent studies have focused on the sole use of exosomes as a cell-free therapeutic approach in cardiovascular repair models (Barile et al., 2014; L. Chen et al., 2013; Gallet et al., 2017; Santoso et al., 2020; Yao et al., 2021a). This is because of the heterogenous composition and their ability to transfer functional proteins, metabolites, and nucleic acids to recipient cells (Gurung et al., 2021a; Y. Zhang et al., 2020). Exosomes are nanosized vesicles that can be secreted from several cell types (Teng & Fussenegger, 2021). There are now recommended set guidelines for extracellular vesicle studies provided by ISEV. Some of them include extracellular vesicle isolation, concentration, characterization, and nomenclature (Théry et al., 2018a; Witwer & Théry, 2019). Extracellular vesicle is the general nomenclature based on recommendations from ISEV. Measurable defined

characteristics such as origin of cell type, size, density, function, molecular markers such as CD63, CD81, CD82 are used to characterize these nanoparticles (Théry et al., 2018b).

Exosomes, which are of endosomal origin, are composed of a single membrane and can range from 30 nm up to 200 nm with an average of 100 nm in diameter (Kalluri & LeBleu, 2020; Pegtel & Gould, 2019; Yi et al., 2020a). Variable exosome sizes have been identified, even when isolating vesicles from a single cell line (Pegtel & Gould, 2019). Exosomes contain variable proportions of genetic information which can be incorporated to the recipient cell (Danac et al., 2021; Narang et al., 2022). Current information on the genomic profile of exosomes derived from CPCs can be influential for determining molecular mechanisms mediating cardiac repair.

Proteomic profiling identified pregnancy-associated plasma protein A (PAPP-A) highly enriched on the exosomal membrane of human CPCs, which activates ERK1/2 and Akt pathways in cardiomyocytes, leading to reduced apoptosis and improved ventricular function after ischemia/reperfusion (Barile et al., 2018). In a recent study using gene expression qPCR array, human CPCs under physoxia showed an increase in extracellular vesicle secretion and minimal changes in cellular expression of hypoxia related genes (Dougherty et al., 2020). Physoxia is described as 5% O_2 saturation, the estimate of an *in vivo* scenario of tissue oxygenation; most tissue is 3.4% to 6.8% concentrated with O_2

(Dougherty et al., 2020). Several miRNAs which are key for cardiac repair have been identified in CPCs and other stem cell-derived exosomes. These miRNAs can regulate cell differentiation, proliferation, angiogenesis, and inhibit apoptosis and fibrosis (Gray et al., 2015; reviewed in Thej & Kishore, 2021; reviewed in Wang et al., 2019). A table summarizing important miRNAs and transcripts found within exosomes derived from stem cells is found below (Table 1)

Table 1. Differentially Expressed miRNAs and Transcripts in Exosomes Derived from Stem Cells

	Source	Content	miRNA Target or Function	Reference
Cardiovascular Progenitor Cells (CPCs)	Human CPCs	miR-210, 132	miRNA-210 targets ephrin A3 and PTP1 and inhibits apoptosis *in vitro*; miRNA-132 targets RasGap-p120 to inhibit apoptosis and enhance tube formation *in vitro*	(Barile et al., 2014)
	Sca-1+ murine CPCs	miR-21	Anti-apoptosis of cardiomyocytes *in vitro*	(Xiao et al., 2016)
	Hypoxia cultured murine CPCs	miR-17, 210	Pro-angiogenic and contributes to improved cardiac function *in vivo*	(Gray et al., 2015)
Cardiosphere-derived cells (CDCs)	Human CDCs	miR-4488	Contributes to anti-apoptotic effects *in vitro*	(Y.-N. Lin et al., 2021)
	Hypoxia cultured human CDCs	miR-126, 130a, 210	Pro-angiogenic	(Namazi et al., 2018)
	Human CDCs	miR-181b	miR-181b targets PKCδ to mediate macrophage polarization *in vitro*	(de Couto et al., 2017)
	Human and Swine CDCs	miR-126, 132, 146a, 181b, 210, 451	Anti-fibrosis, anti-apoptosis, pro-angiogenic functions; miR-146a reduces myocardial inflammation and apoptosis	(Hirai et al., 2020)
	Human CDCs	miR-21, 146a	miR-21 can reduce myocardial apoptosis by modulation of AKT-pathways, PDCD4, and FasL. miR-146a can repress IRAK1 and TRAF6	(Vandergriff et al., 2018)
Mesenchymal Stem Cells (MSCs)	Murine MSCs	miR-182	Improves cardiac function, reduces myocardial infarction size, inflammation, and cell pyroptosis *in vivo*	(Yue et al., 2022)
	Murine MSCs	miR-29, 24	Reduces fibrosis and inflammation and improves cardiac function *in vivo*	(Shao et al., 2017)

	Hypoxia cultured murine MSCs	miR-125b	Suppresses proapoptotic genes p53 and BAK1 which contributes to anti-apoptotic function *in vitro and in vivo*	(L.-P. Zhu et al., 2018)
	Murine MSCs	miR-25	Decreases pro-apoptotic proteins and EZH2 which leads to cardiomyocyte survival and suppresses inflammation *in vitro* and *in vivo*	(Peng et al., 2020)
	Rat bone marrow-derived MSCs	miR-144	Targets PTEN/AKT signaling and contributes to anti-apoptotic effect on cardiomyocytes in hypoxic conditions	(Wen et al., 2020)
	Mouse bone marrow-derived MSCs	miR-181, 223, 124, 146a, 182, 125a, 103, 221, 133, 150, 21, 22	miRNAs are involved in inflammation modulation and/or cardiac repair; miR-182 in exosomes has an anti-inflammatory effect *in vivo*	(J. Zhao et al., 2019)
	Hypoxia cultured human MSCs	miR-26a	Can target and suppress GSK3β and p-β-catenin to reduce Ischemia-reperfusion injury	(H. Park et al., 2018)
Induced Pluripotent Stem Cells (iPSCs)	Murine iPSCs	miR-17-92 cluster, 19b, 20a, 126, 130, 210, 292, 293, 294, 295, let-7, 145, 302a, VEGF-C, BMP-4, PDGFα, PDGFs, TDGF1, FGFs, IGF-2, and CTGF	Involved in angiogenesis, adaptation to hypoxic stress, regulation of cell cycle, mammalian development, aging, late developmental timing, regulation of cellular proliferation, differentiation, apoptosis, maintenance of self-renewal and pluripotency, and proteins involved in stimulating cardiomyogenesis and proliferation	(Adamiak et al., 2018)
	Human iPSC-derived CPCs	miR-92a, 24, 93, 20b, 107, 26a, 16, 130b	Regulation of inflammation	(Lima Correa, El Harane, Gomez, et al., 2021)
Other	Adult murine cardiac stem cells	Ccna2, miR-182, 183, 96, 296, 298	Positively regulates cell cycle, proliferation, and self-renewal	(Scalise et al., 2021)
	Human adipose-derived stem cells	miR-221, 222	Targets PUMA and ETS-1 proteins, decreases apoptosis and hypertrophy-related proteins *in vivo*	(Lai et al., 2020)
	Mice-derived cardiomyocyte	miR-92a	Essential for activation of cardiac myofibroblasts *in vitro* and *ex vivo*	(X. Wang et al., 2020)

Functional Studies with the Use of Stem Cell-derived Exosomes

Functional studies with the use of stem cell-derived exosomes have provided information on variable applications in cardiac repair. Exosomal proteins derived from mesenchymal stem cells reduce the infarcted area by half, inhibit the proliferation and migration of vascular smooth muscle, reduce cardiomyocyte apoptosis, promote angiogenesis, reduce ventricular remodeling, and protect cardiac function (reviewed in B. Liang et al., 2020). Cardiac-derived progenitor cells expressing the early cardiac genes MEF2C, GATA4, and Mesp1 were used to demonstrate the cardioprotective and pro-angiogenic activity of exosomes isolated from these cells (Barile et al., 2018). These CPC-derived exosomes injected intramyocardially after permanent coronary artery ligation reduced infarct scar size and improved cardiac function after four weeks. Most importantly, this study observed a functional difference in cardiac protection between CPC-derived exosomes more than bone marrow-derived mesenchymal stem/progenitor cell-derived exosomes (Barile et al., 2018). Cardiac progenitor cell-derived exosomes reduced cardiomyocyte apoptosis by 53% and were also shown to augment cardiac function after myocardial infarction (Barile et al., 2014; Mol et al., 2017). Patient-derived Sca-1+ CPC-derived exosomes significantly reduced infarct size whereas the parent CPC did not have this effect (Maring et al., 2019). A summary of the functional studies with the use of stem-cell derived extracellular vesicles for applications in cardiac repair is provided in the table below (Table 2).

Table 2. Functional Studies with The Use of Stem Cell-derived Exosomes for Cardiac Repair

	Exosome Cell source	Specific Markers	Differentiation Potential	Dosage of Exosomes	Outcome	Reference
Cardiovascular Progenitor Cells (CPCs)	Human CPCs	c-Kit, CD105, Gata4, MEF2C, and Mesp1	Cardiomyocytes (CMs) and endothelial cells (ECs)	6 x 10^9 particles for neonatal rat *in vivo* model. 10^{11} particles for adult rat *in vivo* model.	Stimulated adult and neonatal cardiomyocyte cycling *in vivo*, improved ejection fraction and reduced scar size in adult rat after myocardial infarction	(Balbi et al., 2021; Smith et al., 2007)
	Human CPCs	c-Kit, CD105, Gata4, MEF2C, Mesp1, CXCR4, and PPAP-A	CMs, ECs, adipogenic (AD), chondrogenic (CH), and osteogenic (OS)	2 x10^{11} particles	Significantly reduced infarct size and improved left ventricle ejection fraction *in vivo*	(Ciullo et al., 2019; Smith et al., 2007)
	Human CPCs	c-Kit, CD105, Gata4, MEF2C, Mesp1, and PPAP-A	CMs, ECs, AD, CH, and OS	10^{11} exosomes	Reduced scar size and improved left ventricular function *in vivo*	(Barile et al., 2018; Smith et al., 2007)
	Adult human CPCs	CD73, -90, -105, Gata4, Tbx5, Tbx18, and Mesp1	None reported	3x10^7 particles/ mL for *in vitro*, 1x10^{11} exosomes for *in vivo* study	Inhibited apoptosis and promotes angiogenesis *in vitro*; improved cardiac function *in vivo*	(Andriolo et al., 2018)
	Human CPCs	CD13, -90, -44, Tbxt, Tbx18, -5 MEF2C, Gata4, and Mesp1	CMs, ECs, AD, CH, and OS	3 doses of 3x10^{10} particles	*In vivo* prevention of Dox/Trz-induced myocardial fibrosis, left ventricular disfunction, and attenuated inflammation	(Milano et al., 2020)
	Human CDCs	c-Kit, Sox2, Nanog, and MHC-I	CMs, smooth muscle cells (SMs) and ECs	16.5 x10^{11} particles	Decreased scar size and improved left ventricular ejection fraction in vivo	(Gallet et al., 2017; T.-S. Li et al., 2010)

Cardiosphere-derived cells (CDCs)	Human CDCs	CD90 and CD105	CMs	6 x10^9 exosomes	Reduced fibrosis and scar size, increased cardiomyocyte proliferation and angiogenesis	(Tang et al., 2017;Vandergriff et al., 2018)
	Human CDCs	c-Kit and CD105	CMs, ECs	2.0 x 10^9 extracellular vesicles injected weekly for four consecutive weeks	Reduced cardiac inflammation, improved cardiac function, suppressed arrhythmogenesis in an *in vivo* arrhythmogenic cardiomyopathy murine model	(Y.-N. Lin et al., 2021; Smith et al., 2007)
Mesenchymal Stem Cells (MSCs)	Murine bone marrow-derived MSCs	CD29, -44, and Sca-1	AD, CH, and OS	5.62 x 10^5 exosomes	Decreased infarct size after ischemia reperfusion injury	(Luther et al., 2018)
	Murine bone marrow-derived MSCs	CD44, -105, and Sca-1	None reported	4 x10^9 particles	Inhibited inflammation, reduced cardiomyocyte apoptosis and fibrosis, enhanced vasculogenesis and restored cardiac function *in vivo*	(X. Wang et al., 2018)
	Murine bone marrow-derived MSCs	None reported	Can differentiate into muscle, fat, bone, and cartilage cells, no *in vitro* or *in vivo* data reported	5.8 x10^{12} particles	Reduced myocardial remodeling *in vivo* and reduced cellular apoptosis *in vitro*	(Ou et al., 2020)
	Mouse cardiac MSCs	CD44, -105, -140, Sca-1, and Gata4	SMs	1 x10^{12} particles	Improved cardiac function, decreased fibrosis, and increased neovasculogenesis *in vivo*	(Xuan et al., 2020)
	Wharton's Jelly-derived MSCs	CD90 and CD105	None reported	1 x 10^{11} particle injections administered weekly: 6 total doses	Decrease in infarct size, improved ejection fraction, cardiac performance, afterload, contractility and lusitropy	(Bellio et al., 2022)

	MSCs	CD31, -34, -90, -105, and c-Kit	None reported	1 x10^8 exosomes in a hyaluronic acid hydrogel	Reduced left ventricular chamber size and preserved wall thickness *in vivo*	(Cheng et al., 2022; Qiao et al., 2019)
Induced Pluripotent Stem Cells (iPSCs)	Human IPSC-derived CPCs	Islet-1, MEF2C, KDR, Gata4, PDGFR- α, Nkx2.5	CMs, ECs, and SMs	1 x10^{10} particles	No humoral response to *in vivo* treatment, pro-inflammatory monocytes and cytokines were decreased *in vivo*	(Drowley et al., 2016; Lima Correa, El Harane, Gomez, et al., 2021)
	Human IPSC-derived CPCs	Islet-1, MEF2C, KDR, Gata4, PDGFR- α, Nkx2.5	CMs, ECs, and SMs	1 x 10^{10} (± 3,000) particles	Failed to trigger cardiomyocyte proliferation but, decreased infarct size and fibrosis *in vivo*	(Lima Correa, El Harane, Desgres, et al., 2021)
	Human MSC-derived IPSC-differentiated cardiomyocytes	Nanog, Oct 3/4, Sox2	IPSCs into CMs	400 x 10^8 exosomes	Enhanced cardiomyocyte survival *in vitro* and cardiac function *in vivo* and induces transcriptional changes to the peri-infarct region by impacting mTOR signaling	(Santoso et al., 2020)
	Human IPSCs and IPSC-derived cardiomyocytes	None reported	CMs	3x10^{10} extracellular vesicles	IPSC-derived cardiomyocyte extracellular vesicles reduced infarct size, hypertrophy, apoptosis and arrythmias	(B. Liu et al., 2018)
	Human IPSC-derived CPCs	Islet-1, MEF2C, KDR, Gata4, PDGFR- α, Nkx2.5	CMs, ECs, SMs	3 x 10^{10} extracellular vesicles for *in vivo* myocardial infarction model	Significantly improved cardiac function, decreased left ventricular volumes, increased left ventricular ejection fraction, and induction of genes associated with cardiac functional improvement	(El Harane et al., 2018a)

Other	Human adipose-derived stem cells	Not reported	ECs	1.3 x 10^{10} particles in hindlimb Ischemia model, for myocardial infarction model two 2.2 x 10^{7} injections, and intravenous 4.3 x 10^{8} particle injections at 7, 14, 21 days post-surgery	Promote angiogenesis and arteriogenesis in ischemic hindlimb *in vivo* while also improving cardiac function, reducing infarct size, and promoting angiogenesis in myocardial infarction *in vivo* model	(Kang et al., 2016; D. Zhu et al., 2022)
	Murine endothelial progenitor cells	DiLDL, VEGFR2, and CD34	Can differentiate into *de novo* vasculature; no data reported	1.87 x 10^{11} particles/mL for *in vitro* studies, 9.33x10^{10} particles/mL for *in vivo* studies,	Proangiogenic effect in vitro, improved left ventricular contractility and structural integrity *in vivo*	(Atluri et al., 2014; Chung et al., 2020)
	Human embryonic stem cell-derived CPCs	SSEA1, Mesp1, MEF2C, Islet-1, Gata4, and Nkx2.5	CMs, ECs, and SMs	Around 485 ± 827 x 10^{8} particles in the normoxia group and 457 ± 927 x10^{8} particles for hypoxia treatment	Hypoxia and normoxia improved cardiac function, reduced scar size, promoted angiogenesis and reduced fibrosis	(J. Wang et al., 2019; Q. Wu et al., 2020)
	Human and murine epicardial cells	Murine epicardial cells: Wt1	Murine epicardial cells can differentiate into SMs	1.58 x10^{8} particles for *ex vivo*, 10^{8} for *in vivo*	Promote cell cycle activity *ex vivo* and increased proliferation *in vivo*	(Austin et al., 2008; del Campo et al., 2021)

CMs: Cardiomyocytes,
ECs: Endothelial cells,
SMs: Smooth muscle cells
AD: Adipogenic,
CH: Chondrogenic
OS: Osteogenic

Islet-1+ Expressing Progenitors as a Source for Cardiac Repair

Neonatal Islet-1+ cardiovascular progenitor-derived exosomes may be a more advantageous source for cardiac repair since the neonatal heart is capable of regeneration during a temporary postnatal period (Weinberger & Eschenhagen, 2021). In a study using neonatal mice, cardiac regeneration was observed after ventricular resection, but the regenerative window for mice is soon diminished after birth and measured within three to seven days (Velayutham et al., 2019). Furthermore, in an *in vivo* myocardial infarction model using pigs, the regenerative potential is observed during the first two days after birth and potential is lost a few days after (Ye et al., 2018; W. Zhu et al., 2018). This regenerative window is in part achieved due to YAP1 signaling (Mia & Singh, 2019; J. Wang et al., 2018). As the critical effector of the Hippo signaling pathway, YAP1 is sufficient to promote mitosis in cardiomyocytes and can activate surrounding tissue by means of secreted signals through downstream targets (Gong et al., 2021a; Mugahid et al., 2020a). The potential use of Islet-1+ neonatal cardiac progenitor-derived exosomes for the purpose of augmenting YAP1 expression and activating proliferation in patient-derived adult cardiac progenitors which have reduced regenerative ability is the focus of the work described in this thesis.

CHAPTER TWO

MATERIALS AND METHODS

In Vitro Exosome Isolation and Application

Isolation and Culture of Human Neonatal Islet-1+ and Adult Cardiovascular Progenitor Cells

The Institutional Review Board of Loma Linda University approved the protocol for use of tissue that was discarded during cardiovascular surgery, without identifiable private information, for this study with a waiver of informed consent. Human neonatal Islet-1+ cardiac progenitor and adult cardiac progenitor cell clones were previously isolated from discarded surgical cardiovascular tissue (Fuentes et al., 2013b) and were available for use in this study. Briefly, discarded atrial tissue from human neonates and adults was cut and digested in a collagenase solution (Roche Applied Science, Indianapolis, IN, USA). In order to isolate cardiovascular progenitors, this solution was strained through a 40 μm cell strainer. Clonal populations of cells were established by a limiting dilution at a concentration of 0.8 cells per well which were further expanded. Human CPC clones used in the current project were cultured in growth media that included 10% fetal bovine serum (FBS) or exosome-depleted 10% FBS (Genesee Scientific, San Diego, CA, USA), Medium 199 (Thermo Fisher Scientific, Waltham, MA, USA), 100 μg/mL Penicillin-Streptomycin (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 22% EGM-2 Endothelial Cell Growth Medium-2

BulletKit (Lonza, Basel, Switzerland), and 1.0% minimum essential medium non-essential amino acids solution (Life Technologies by Thermo Fisher Scientific, Waltham, MA, USA).

Preparation of Exosome Depleted Fetal Bovine Serum

Extracellular vesicles were removed from the serum used for cell culture, using an approach that removes approximately 95% of FBS-derived extracellular vesicles containing RNA (Shelke et al., 2014; Théry et al., 2006). Briefly, 10% fetal bovine serum (Genesee Scientific, San Diego, CA, USA) was subjected to ultracentrifugation at 100,000 x g for 18 hours at 4 °C then filtered with a .22μm filter to reduce contamination and stored in -18 °C until needed.

Collection, Isolation and Quantification of Exosomes from Neonatal Islet-1+ Cardiovascular Progenitor Conditioned Media

Neonatal Islet-1+ cardiovascular progenitor cells were cultured in 6 well 0.1% gelatin-coated plates and with exosome-depleted 10% FBS-prepared medium. Neonatal cardiovascular progenitor cells were grown in exosome-depleted media and were incubated at 37 °C with 5% CO_2 and 95% oxygen until 90% confluency in order to collect conditioned media. Conditioned media was saved in -80 °C and thawed at room temperature prior to exosome isolation based on previous recommendations (Bojmar et al., 2021; Trummer et al., 2009).

Total Exosome Isolation from Cell Culture Media

Total Exosome Isolation Reagent was used according to manufacturer's instructions (Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA). Briefly, conditioned media was centrifuged at 2,000 x g for 30 minutes and suspended with 0.5 volume of the Total Exosome Isolation reagent (10mL of media: 5 mL of reagent). The solution was incubated overnight at 4°C, then subjected to a final centrifugation of 10,000 x g for 1 hour at 4 °C. The supernatant was aspirated and discarded, and the pelleted exosomes were resuspended in 200 μL of PBS (Genesee Scientific, San Diego, CA, USA). A dilution of 3:1000 was prepared for Nanosight analysis and stored at -20 °C or -80 °C for either short term or long term storage, as recommended (Bertokova et al., 2022; Bojmar et al., 2021).

Differential Ultracentrifugation of Cell Culture Media for Exosome Isolation

Differential ultracentrifugation was performed to separate particles by sedimentation dependent on size and density through sequential centrifugal speeds. The procedure was followed as previously described with slight modification (Livshits et al., 2015; Sidhom et al., 2020). Briefly, conditioned media collected from neonatal cardiovascular progenitors was subjected to a centrifugal force of 2,000 x g for 30 minutes for removal of cellular debris. The pellet was discarded, and the supernatant went through a subsequent centrifugal force of 10,0000 x g for 30 minutes at 4 °C to remove apoptotic bodies and contaminating proteins. The pellet was discarded, and the supernatant was

subjected to a final centrifugation of 100,000 x g for 2 hours at 4°C. The supernatant was aspirated, and the pellet was resuspended with 203 μL of PBS (Genesee Scientific, San Diego, CA, USA). A dilution of 3:1000 was prepared for Nanosight and saved in -20 °C or -80 °C.

Exo-Quick-TC ULTRA EV Isolation Kit for Cell Culture Media

The Exo-Quick TC ULTRA EV isolation kit was used to purify exosomes from the media of neonatal cardiovascular progenitors according to manufacturer's instructions (System Biosciences, Palo Alto, CA, USA). Briefly, conditioned media was centrifuged at 3,000 x g in order to remove cellular debris. The supernatant was resuspended with the ExoQuick-TC reagent using a ratio of 5mL of conditioned media to 1mL of reagent and was incubated overnight at 4 °C. Subsequently, the solution was centrifuged for 10 minutes at a speed of 3,000 x g at room temperature. The supernatant was aspirated to leave the precipitated exosomes for further collection. The pellet was resuspended with 200 μL of Buffer B, followed by addition of 200 μL of Buffer A and resuspension. The solution was loaded onto a prepared isolation column and placed on a rotating shaker for 5 minutes at room temperature. The purified exosomes were eluted at a speed of 1,000 x g for 30 seconds, and this step was repeated. A dilution of 3:1000 was prepared for Nanosight preparation and saved in -20 °C or -80 °C.

Nanoparticle Tracking Analysis

Analyses of particle size and concentration of exosomes isolated from neonatal cardiovascular progenitor cell conditioned media was performed using Nanosight NS300 instrument (Malvern Panalytical, Malvern, UK) and Nanoparticle Tracking Analysis (NTA) software (version 3.4; Malvern Panalytical, Malvern, UK). Exosome samples diluted in PBS (3:1,000) were thawed at room temperature. Samples were vortexed and sonicated for 30-45 seconds before injection. Five videos of one sample determined mean size and concentration of particles: syringe pump speed, 30.

RNA Sequencing and Transcriptomic Analysis of Adult and Neonatal Islet-1+ CPC-derived Exosomes

RNA samples were previously extracted and purified from exosomes derived from adult and neonatal Islet-1+ cardiovascular progenitors. RNA samples were sent to the PrimBio Research Institute (Exton, PA, USA) for transcriptome analysis following the same protocol mentioned previously from our lab (Camberos et al., 2021). Briefly, rRNA was removed from total RNA samples with a rRNA removal kit from Illumina (San Diego, CA, USA). Ion Total RNA-Seq Kit v2 (Thermo Fisher Scientific, Waltham, MA, USA) was used to assemble sequencing libraries. Prior to PCR amplification, nucleic acid binding beads (Ambion, Austin, TX, USA) were used to purify the cDNA library. Agilent dsDNA High Sensitivity kit (Agilent, Santa Clara, CA, USA) was used to test

quality of libraries. Samples were enriched with a Ion OneTouch ED instrument and an Ion PI ™ Hi-Q™ OT2 200 Kit (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was performed with an Ion Proton sequencer (Thermo Fisher Scientific, Waltham, MA, USA) and a species-specific protocol. Sequence files were aligned to the human genome and quality of sequence files was performed using the Strand NGS program. Quantification and normalization of aligned reads were performed using the DEseq algorithm within the Strand NGS program. The Audic-Claverie test and the Benjamini-Hochberg correction test were used for statistical analysis. Significance was determined using a 2.0-fold change minimum cutoff.

miRNAs which were predicted as upregulated or downregulated and showing greater than a 2.0 fold change in neonatal CPC-derived exosomes when compared to adult CPC-derived exosomes were uploaded to DIANA-miRPath v3.0 bioinformatics software (Vlachos et al., 2015). A KEGG analysis was performed on the dataset based on predicted targets of uploaded miRNAs. Categories specific to pathways involving development, proliferation, cell-cycle, or Hippo signaling pathway and only categories with a p-value of < 0.05 were reported. Total gene transcripts that were predicted to be upregulated and showing greater than a 2.0 fold change were then uploaded onto Ingenuity Pathway Analysis (IPA) (Qiagen, Valencia, CA, USA). A core analysis was performed with a parameter of human cells only was set for the anaylsis. Categories specific to pathways involving exosome biogenesis, development,

proliferation, cell-cycle, or Hippo signaling pathway and with a p-value of < 0.05 were reported.

Treatment of Human Adult Cardiac Progenitor Cells with Exosomes Isolated from Neonatal Cardiovascular Progenitors

Human adult cardiovascular progenitor cells were grown on CPC growth media until exosome addition. Adult CPC clones received concentrations of 5 – 6 $x10^{10}$ exosomes isolated from human neonatal Islet-1+ CPC clones or similar volume of exosome-depleted media as a control. Cells underwent a 72-hour incubation at 37 °C with 5% CO_2 and 95% Oxygen before subsequent experiments.

Purification of RNA and Reverse Transcriptase Quantitative PCR

Adult treated and non-treated CPCs received 700 μL of QIAzol® reagent (Qiagen, Valencia, CA, USA). Total RNA was purified by using an RNeasy mini kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. RNA quality was validated with a gel electrophoresis using a 1% agarose gel and high mass DNA ladder (Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA) (E. J. Park et al., 1996). 1 μg of RNA was used to prepare cDNA with Superscript III (Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcriptase quantitative PCR was performed using iTaq™ Universal SYBR® Green Supermix (Bi-Rad, Hercules, CA, USA). Bio-Rad CFX96 Touch Real-Time

PCR Detection System was used to perform all RT-qPCR experiments (Bi-Rad, Hercules, CA, USA). PCR plates were run with the following settings 94 °C for 10 minutes, 94 °C for 15 seconds, 56 – 58 °C (depending on the primer) for 60 seconds, and 72 °C for 30 seconds, repeated for 45 cycles. Primers of our genes of interest were constructed using National Center for Biotechnology Information (NCBI) Primer-BLAST and are shown in Table 3. Reverse transcriptase quantitative PCR products were visualized using a 1%-2% agarose gel electrophoresis and a low mass DNA ladder to ensure that the correctly sized transcripts were amplified (Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA).

Table 3. Primer Sequences Used for RT-qPCR; (5’ to 3’ from left to right)

Human Primers	**Sequence**
bACTIN - FWD	TTT GAA TGA TGA GCC TTC GTC CCC
bACTIN - REV	GGT CTC AAG TCA GTG TAC AGG TAA GC
CCNA2 - FWD	AGG AAA GCT TCA GCT TGT GG
CCNA2 - REV	TTG AGG TAT GGG TCA GCA TC
CCND1 - FWD	TTC ACA GAG CGC CAG CCA GC
CCND1 - REV	CTT GGG AGC GGC GGC AAG AA
CREB – FWD	AGG TGT AGT TTG ACG CGG T
CREB - REV	GGA CTT GAA CTG TCT GCC CA
CTGF - FWD	CAC CCG GGT TAC CAA TGA CA

CTGF - REV	TCC GGG ACA GTT GTA ATG GC
ERBB4 - FWD	TTC AGG ATG TGG ACG TTG CC
ERBB4 - REV	GGG CAA ATG TCA GTG CAA GG
MYC – FWD	AAG ACA GCG GCA GCC CGA AC
MYC – REV	TGG GCG AGC TGC TGT CGT TG
PIK3CA - FWD	AAC AAT GCC TCC ACG ACC AT
PIK3CA - REV	TCA CGG TTG CCT ACT GGT TC
RelA - FWD	GCG AGA GGA GCA CAG ATA CC
RelA - REV	GGG GTT GTT GTT GGT CTG GA
SOX2 - FWD	AAC CAG CGC ATG GAC AGT TA
SOX2 - REV	GAC TTG ACC ACC GAA CCC AT
YAP1 - FWD	TCC CAG ATG AAC GTC ACA GC
YAP1 - REV	TCA TGG CAA AAC GAG GGT CA

Protein Purification from Human Adult CPCs after Exosome Treatment for Western Blot

Following a 72-hour neonatal Islet-1+ CPC-derived exosome treatment or control treatment, the adult CPCS were aspirated to remove any medium, washed with cold PBS (Genesee Scientific, San Diego, CA, USA) and were incubated with cold trypsin (Thermo Fisher Scientific, Waltham, MA, USA). Trypsinized cells were placed on ice until all cells became detached. Adult exosome and control samples received a protein lysis buffer solution consisting of RIPA buffer, 0.5M EDTA, protease inhibitor cocktail, sodium orthovanadate,

and sodium fluoride and were agitated for 1 hour at 4 °C. The samples were then subjected to centrifugation at 14,000 x g and aliquoted for use. Protein concentration was quantified using the Micro BCA Protein Assay Kit (ThermoFisher Scientific, Waltham, MA, USA). Simple Wes by Protein Simple (ProteinSimple, San Jose, CA, USA) is an automated gel-free western blotting system and was used following the manufacturer's instructions to analyze protein levels in adult CPCs. Antibodies used for western blot are shown in Table 4 below (Cell Signaling Technology, Danvers, MA, USA).

Table 4. Antibodies Used for Western Blot

Antibody	**Species**	**Antibody Dilution**	**Size (kDa)**	**Sample Used**	**Catalog No.**	**Manufacturer**
YAP1	Rabbit	1:200	65-78	0.4 mg/μL	D8H1X	Cell Signaling Technology
Phosphorylated YAP1	Rabbit	1:200	65-78	0.4 mg/μL	D9W2I	Cell Signaling Technology
beta-Actin	Mouse	1:50	45	0.4 mg/μL	8H10D10	Cell Signaling Technology

Statistical Analysis

Data was analyzed using Microsoft Excel and PRISM software programs and was reported as mean +/- standard error. Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method and P values < 0.05 were deemed significant (Livak & Schmittgen, 2001). Actin was used to normalize genes and proteins of interest.

CHAPTER THREE

RESULTS

Transcriptomic Analysis of Neonatal Cardiovascular Progenitor Cell-derived Exosomes and Their Functional Application in Adult CPCs

The Transcriptome of Islet-1+ Neonatal Cardiac Progenitor Cell-derived Exosomes

The transcriptome of human neonatal Islet-1+ cardiac progenitor cell-derived exosomes was compared with the transcriptome of exosomes derived from human adult CPC clones. miRNAs which were differentially expressed, showing greater than a 2.0 fold change, were uploaded into DIANA-miRPath v3.0 bioinformatics software to identify which KEGG pathways were predicted to be regulated by the exosome content of neonatal CPC.

Figure 1A, identifies the pathways predicted to be regulated by miRNAs identified in neonatal CPC-derived exosomes. The most significant pathways found were the Hippo signaling pathway and cell cycle. These two pathways are critically important for cardiac regeneration. Previous studies have shown that the Hippo signaling pathway is critical for cardiomyocyte proliferation in the postnatal heart through activation of the downstream effector YAP1 (Z. Lin & Pu, 2014). In Figure 1A, there are an estimated 117 genes targeted by about 56 miRNAs identified in the neonatal exosome cargo regulating the Hippo signaling pathway. For example, miR-31 and miR-221 are able to indirectly activate YAP1

transcriptional activity through suppression of LATS (Aguennouz et al., 2020; Mitamura et al., 2014). In addition, miR-221 is capable of targeting cyclin-dependent kinase inhibitors (CDKI) CKKN1B/p27 and CDKN1C/p57. Consequently, miR-221 can promote proliferation through modulation of cell-cycle dependent genes (Fornari et al., 2008). The manipulation of the Hippo signaling pathway can influence YAP1-mediated de-differentiation of adult cardiomyocytes (Y. Zhu et al., 2021a). De-differentiation is necessary for cell cycle re-entry, and is a key step in activating cardiac regeneration (Y. Zhu et al., 2021b). YAP1 signaling via exosomes can drive tissue repair in the infarcted heart by initiating a de-differentiation step in surrounding cardiac myocytes.

In addition to the Hippo signaling pathway, several other significantly impacted pathways listed in Figure 1A are also involved in regeneration and in development of the human heart (Hesse et al., 2018; H. Li et al., 2021; Ozhan & Weidinger, 2015; Wadugu & Kühn, 2012). These pathways include cell cycle, Wnt signaling, ERBB signaling, and Notch signaling. These pathways interact with the Hippo signaling pathway and mediate YAP1 transcriptional activity (Aharonov et al., 2020; Flinn et al., 2020; F. Zhu et al., 2021). For example, Notch and Hippo signaling pathways are able to promote the expression of neuregulin (Artap et al., 2018; K. Zhang et al., 2012). Neuregulin plays a role in ERBB signaling by interacting directly with ERBB2/ERBB4 receptors and is able to stimulate cardiomyocyte proliferation (Vujic et al., 2020). Regulation of the actin cytoskeleton, TGF-beta signaling pathway, and Focal adhesion are

pathways which are influenced by the extracellular matrix. Mechanical or signaling cues activate YAP1 transcriptional activity (Morice et al., 2020; Seo & Kim, 2018). mTOR signaling has an improtant role in regulation of cellular growth, metabolism, proliferation, and survival (Saxton & Sabatini, 2017). Specifically, mTOR-dependent G1-phase progression of the cellular cycle is possible through S6KI and eukaryotic translation Initiation Factor 4E (eIF4E) (Fingar et al., 2004).

DIANA miRPath is used to analyze miRNAs only. We next uploaded transcripts showing greater than a 2.0 fold change into Ingenuity Pathway Analysis. This was done to identify biological relationships between transcripts and miRNAs in the neonatal CPC exosome content. In Figure 1B, several additional signaling pathways were identified as significantly impacted, including CREB signaling. The cyclic adenosine monophosphate response element-binding protein (CREB) is able to promote the transcription of YAP1 by binding to the YAP1 promoter (Han, 2019). Several additional upstream components that have a significant impact on Hippo signaling include mechanical cues, G-protein coupled receptor signaling and oxidative stress (Luo & Yu, 2019; Meng et al., 2016). Calcium signaling, cAMP-mediated signaling, growth hormone signaling, GP6, and protein kinase A signaling are all pathways critically necessary for physiological cardiac function and repair.

Calcium signaling is required for early cardiac development and is necessary for the proliferation of cardiomyocytes in the embryonic heart

(Chernyavskaya et al., 2012; Porter Jr. et al., 2003). In addition, based on genetic analysis in early heart development, calcium signaling, paracrine communication and transcriptional signaling are major processes involved in cardiac morphogenesis (Chernyavskaya et al., 2012). cAMP utilizes PKA to activate the epidermal growth factor signaling pathway and ERK1/2 signaling to actvate cellular proliferation (Kiermayer et al., 2005). Growth hormone signaling has been shown to be necessary in liver regeneration given that impaired growth hormone signaling led to reduced cellular proliferation (Chia, 2014). Other significant pathways such as SNARE signaling are involved in exosomal biogenesis and secretion (Gurung et al., 2021b). Finally, cell cycle control of chromosomal replication is also found to be a significant pathway which predicts that the cyclin-dependent kinases are activated and being transferred through exosomal platforms to nearby cells and this predictation can be extremely important for cardiac repair.

IPA allows for identification of predicted molecule activity in both upstream and downstream regulation of signaling pathways. This dataset was aligned with the Hippo signaling pathway in Figure 3A. The Molecule Activity Predictor (MAP) indicates that YAP1 is predicted to be able to translocate into the nucleus and interact with transcriptional factors involved with activation of cellular proliferation.

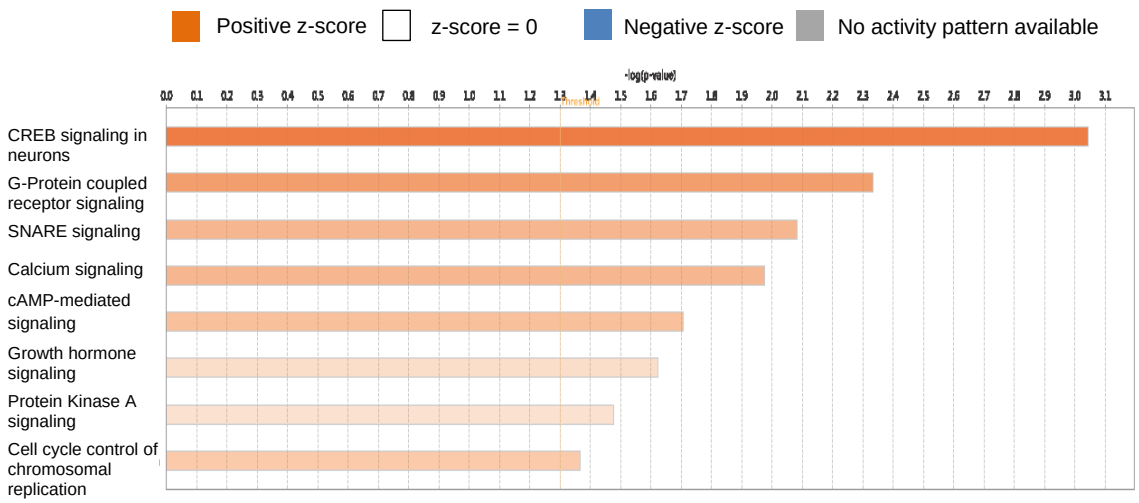
Given that the cell cycle and Hippo signaling pathways are likely to be significantly impacted according to predictions made by the software used to analyze the CPC transcriptome, further experiments were done to functionally

assess YAP1 activation and proliferation. In order to examine whether or not cellular proliferation is induced by the content of neonatal exosomes *in vitro*, we initially isolated Islet-1+ neonatal cardiac progenitor cell-derived exosomes and quantified them by Nanosight.

A

KEGG pathway	p-value	#genes	#miRNAs
Hippo signaling pathway	1.68032939729E-08	117	56
Cell cycle	1.13003361222E-07	100	57
TGF-beta signaling pathway	3.60169051971E-06	64	57
p53 signaling pathway	7.64410236146E-06	60	52
mTOR signaling pathway	9.36969660104E-05	51	50
Signaling pathways regulating pluripotency of stem cells	9.64620797651E-05	104	59
Wnt signaling pathway	0.000402401	100	54
Regulation of actin cytoskeleton	0.001527586	149	54
Focal adhesion	0.001567503	149	59
FoxO signaling pathway	0.001747196	101	58
VEGF signaling pathway	0.002937814	49	44
ErbB signaling pathway	0.004527734	66	49
Apoptosis	0.015545555	65	45
MAPK signaling pathway	0.017086699	173	59
Notch signaling pathway	0.03396616	37	47

B



C

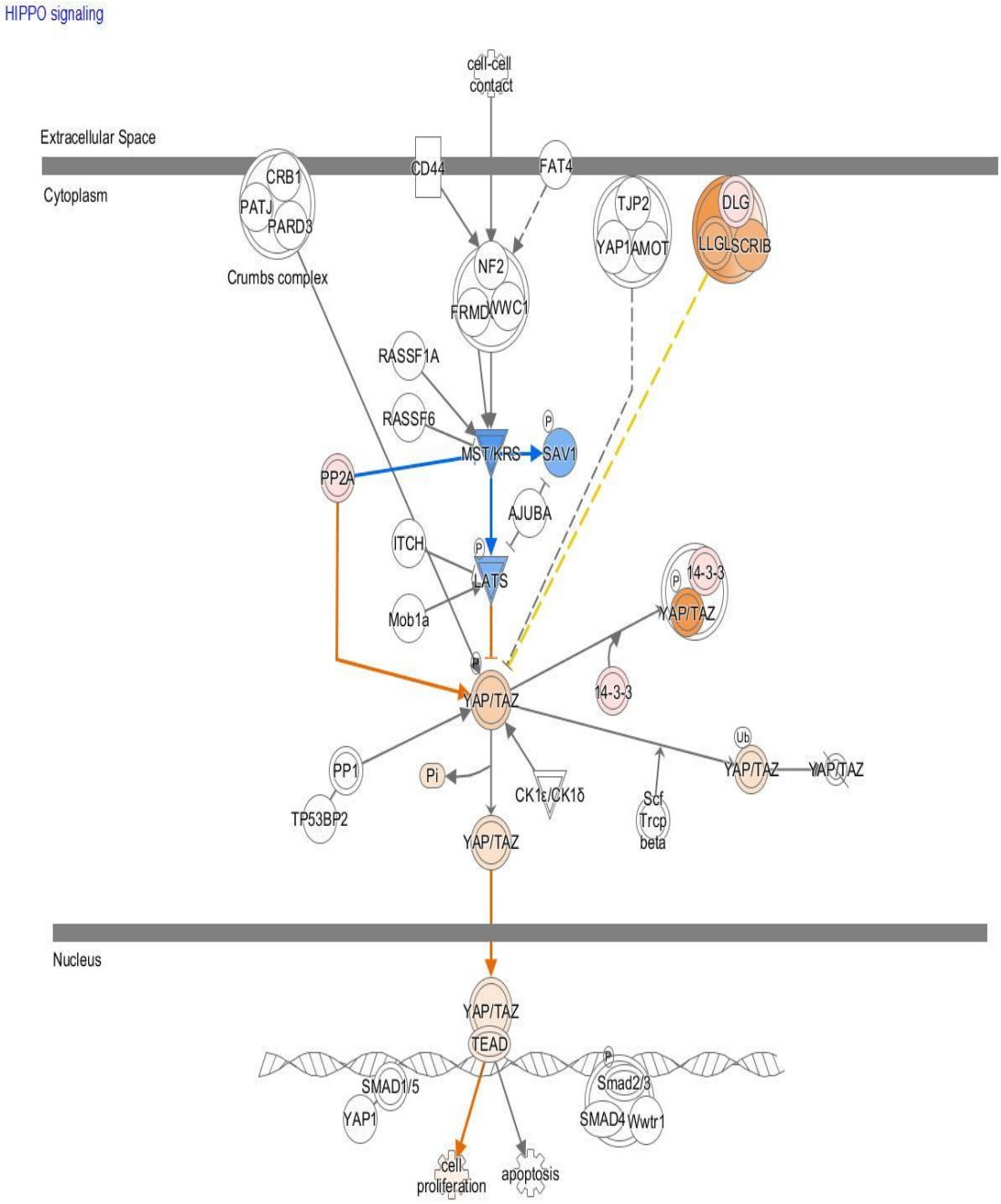


Figure 1. Transcriptomic Analysis Reveals the Predicted Activation of YAP1 and Cell Proliferation by Neonatal Cardiovascular Progenitor Cell-derived Exosomes

(A) KEGG analysis of miRNAs identified by RNA sequencing of Isl-1+ neonatal versus adult CPC-derived exosomes **(B)** Canonical Pathways significantly impacted by upregulated miRNAs and transcripts in the Isl-1+ neonatal CPC exosome content **(C)** The Molecule Activity Predictor tool was used on IPA to predict the biological relationships between upregulated miRNAs and transcripts in relation to the Hippo signaling pathway. The z-score indicates the observed increase in the activation states of biological functions which are impacted by several signaling pathways. Orange z-score describes predicted activation while blue z-score indicates predicted inhibition. A -log(p-value) of 1.3 is considered statistically significant.

Purification of Neonatal Islet-1+ Cardiac Progenitor Cell-derived Exosomes

Exosomes capture an important part of the secretome that mediate functional alternations in recipient cells. Several methods have been used to obtain exosomes with an optimal size range of 30-150 nanometers and up to 200 nanometers in size (Brennan et al., 2020; Xu et al., 2022). Purity, size, and concentration can be influenced by differential isolation methods and culture conditions (Ludwig et al., 2019). Successful isolation of purified functional exosomes would aid in the application of exosomes for cardiac therapy.

No optimal method of exosome isolation exists. Ultracentrifugation utilizes high centrifugal force to extract exosomes from biological fluids based on differential sedimentation rates (H. T. Hu et al., 2021). The limitation of this approach is that ultracentrifugation can negatively impact purity and lacks high exosome yield (Ayala-Mar et al., 2019; Serrano-Pertierra et al., 2019). Moreover, the repeated subjection of nanoparticles to high centrifugal force can damage

exosomal membranes and alter their biological function (Butreddy et al., 2021; J. Chen et al., 2022). We compared ultracentrifugation to commercially available isolation kits to assess functional efficacy and to identify the optimal isolation methods for our proposed study.

Commercial kits contain a precipitation reagent (hydrophilic polymer) which allows for the separation of exosomes from the cell culture medium. Precipitation-based methods preserve biological function but result in low purity after exosome isolation (Ayala-Mar et al., 2019). The ExoQuick-TC ULTRA EV Isolation Kit for Tissue Culture Medium differs from the Total Exosome Isolation kit based on the inclusion of a subsequent purification step after exosome precipitation. The kit provides a “purification column” and has significantly decreased the presence of contaminating proteins such as IgG and albumin in previous studies (Coughlan et al., 2020).

Comparison of different isolation techniques using supernatant isolated from neonatal cardiac progenitor cells allowed us to determine the purity and size distribution of exosomes isolated from Islet-1+ progenitor cell conditioned media. The concentration of exosomes in our samples was performed using Nanosight instrument. Nanosight measures particle size distribution by nanoparticle tracking analysis. Briefly, Nanoparticle tracking analysis uses a specially aligned laser beam to illuminate particles in liquid suspension. The light scattered by each particle is focused onto a microscope and recorded onto a camera (Gardiner et al., 2013). Nanoparticle tracking analysis software tracks the particles moving in

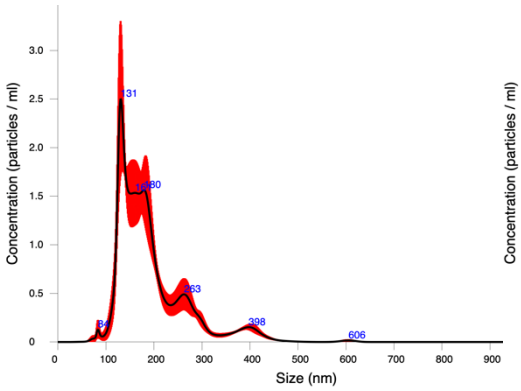
Brownian motion to calculate particle size using the Stokes-Einstein equation (Comfort et al., 2021). Size distribution was determined by five video records for each sample, n=5. Concentration measurements represent mean +/- standard error.

In Figure 2A, Nanoparticle tracking analysis of particles which were isolated by differential ultracentrifugation of the cell culture supernatant of Islet-1+ neonatal cardiovascular progenitor cells revealed a mean particle size of 193 nm and concentrations of $1.32x10^{10}$ +/- $4.7x10^{9}$ with a mode of 131 nm. Figure 2B demonstrates the outcome when Nanoparticle tracking analysis was applied to a sample of isolated particles from this same neonatal cardiovascular progenitor cell clone using the Total Exosome Isolation kit. The results revealed a mean particle size of 180 nm and concentrations of $1.97x\ 10^{10}$ +/- $1.12x\ 10^{9}$ with a mode of 141 nm using this isolation kit. As shown in Figure 2C, the ExoQuick-TC ULTRA-EV Isolation Kit resulted in isolation of particles found with a mean particle size of 176 nm and concentrations of $2.13x\ 10^{10}$ +/- $2.57x\ 10^{10}$ with a mode of 137 nm.

We chose to use the ExoQuick-TC method going forward because the mean and mode particle sizes found within our sample remained under the threshold of exosome characterization, (< 200nm) (Yi et al., 2020b). Additionally, the ExoQuick-TC isolation method had the same starting volume as other exosome isolation methods and resulted in a 10-fold higher yield of exosomes. Given these results, including the extra purification step, we decided to continue

with the ExoQuick-TC method for downstream applications. Now that we had optimized the exosome isolation process for our proposed study, we next examined whether or not exosomes derived from neonatal cardiac progenitor cells can alter the transcriptome of adult cardiac progenitor cells and potentiate a proliferative state.

A



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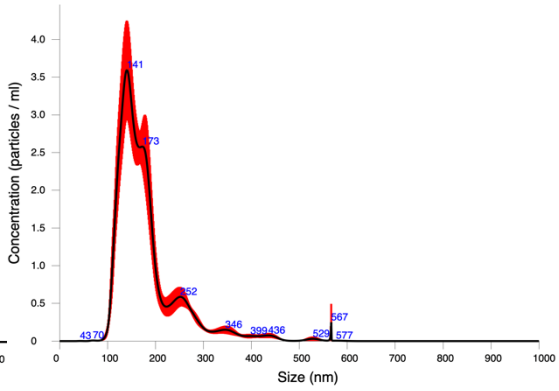

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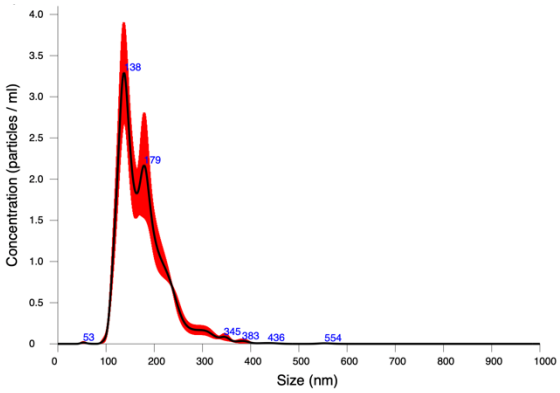


Figure 2. Nanosight Analysis of Exosome Size Using Three Different Methods of Isolation

Nanosight was used to identify the size of extracellular vesicles isolated from neonatal Islet-1+ cardiac progenitor cell clones. Representative graphs of size distribution profile data, n=5 from one sample. **(A)** Nanoparticle tracking analysis of extracellular vesicles isolated using differential ultracentrifugation **(B)** Extracellular vesicles isolated by a precipitation-based Total Exosome Isolation kit by Invitrogen **(C)** Extracellular vesicle isolation and purification by a precipitation and column-based approach using ExoQuick-TC ULTRA-EV Isolation Kit for Tissue Culture Media

YAP1 RNA and Protein Expression is Elevated in Adult Cardiac Progenitor Cell Clones Following a 72-hour Treatment with Islet-1+ Neonatal Cardiac Progenitor Cell-derived Exosomes

We treated adult cardiovascular progenitors with exosomes derived from neonatal Islet-1+ cardiac progenitor cells in order to functionally assess YAP1 activation in the adult CPC. Adult cardiac progenitor cell clones received concentrations of 5 – 6 x 10^{10} exosomes isolated from human neonatal Islet-1+ CPC clones or similar volume of exosome depleted media as a control. RNA was extracted from adult cardiovascular progenitor cells after a 72-hour treatment of neonatal Islet-1+ cardiovascular progenitor cell derived-exosomes. In a different set of samples, protein was collected and isolated from adult CPCs after a 72-hour treatment of neonatal Islet-1+ cardiovascular progenitor cell derived-exosomes. We assessed RNA and protein isolated from the adult CPCs by RT-qPCR and Western Blot analysis, respectively.

In Figure 3A, statistical analysis revealed significantly elevated levels of YAP1 RNA (1.53 ± 0.25 FC, * p = 0.0286) after a 72-hour treatment of neonatal cardiovascular derived exosomes. In Figure 3B, we confirmed the transcript size of the RT-qPCR amplified product, YAP1, by gel electrophoresis (187 bp). Since we saw a significant increase of YAP1 transcripts after neonatal exosome treatment, we quantified the protein levels of YAP1 before and after exosome treatment. We observed a significant increase in YAP1 protein (1.77 ± 0.09 FC, **** p<0.0001) as a result of exosome treatment as shown in Figure 3C. Quantification and visualization of YAP1 protein is shown in Figure 3D.

We observed a significant increase in the ratio of phosphorylated YAP1 relative to the non-phosphorylated YAP1 protein (1.11 ± 0.13 FC, * p= 0.0426) as shown in Figure 3E. Quantification and visualization of phosphorylated YAP1 and Actin protein is shown in Figure 3F. Based on several studies, depending on the phosphorylation site such as S127, phosphorylated YAP1 is still able to accumulate in the nucleus (reviewed in Piccolo et al., 2014a). This finding is consistent with the phosphorylated YAP1 antibody used in this experiment. The accumulation of YAP1 in the nucleus can lead to the interaction of transcription factors and subsequent activation of transcripts involved with proliferation which is necessary for cardiac repair.

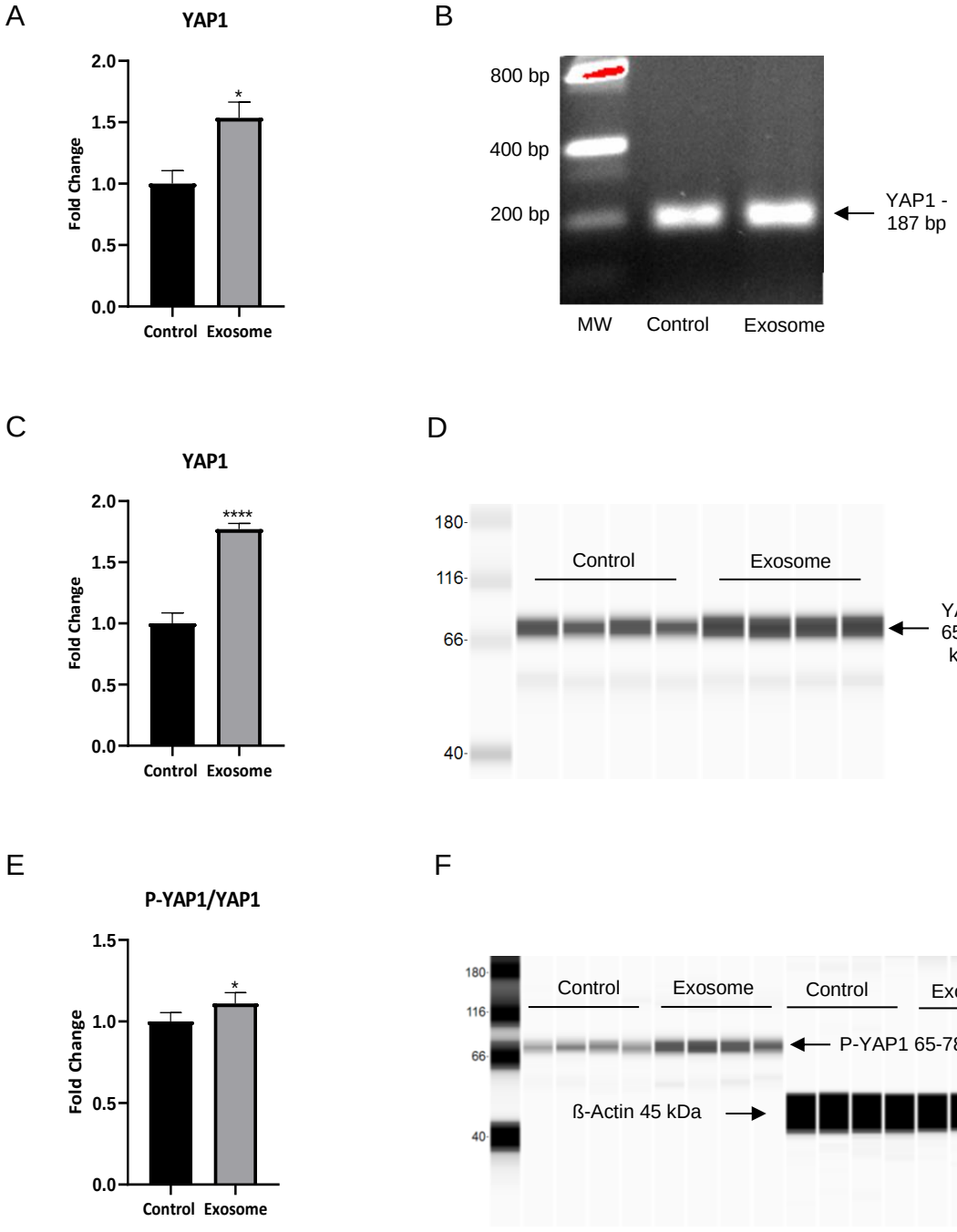

A
YAP1
Fold Change
2.0
1.5
1.0
0.5
0.0
*
Control
Exosome
B
800 bp
400 bp
200 bp
YAP1 -
187 bp
MW
Control
Exosome
C
YAP1
Fold Change
2.0
1.5
1.0
0.5
0.0
****
Control
Exosome
D
180
116
66
40
Control
Exosome
YAP1
65-78
kDa
E
P-YAP1/YAP1
Fold Change
1.5
1.0
0.5
0.0
*
Control
Exosome
F
180
116
66
40
Control
Exosome
Control
Exosome
P-YAP1 65-78 kDa
ß-Actin 45 kDa

Figure 3. Neonatal Cardiovascular Progenitor Cell-derived Exosome Treatment in Adult CPCs Results in Increased YAP1 Transcript and Protein Levels

(A) YAP1 transcript is significantly elevated in the adult CPC following a 72-hour treatment with exosomes isolated from Islet-1+ neonatal cardiovascular progenitor cells as shown by RT-qPCR. **(B)** Gel electrophoresis of the RT-qPCR product YAP1, amplifies a product of the correct size (187 bp). Quantification of protein levels was performed with Protein SimpleWes automated gel-free western systems. **(C)** Analysis of YAP1 protein in the adult CPC following a 72-hour treatment with exosomes isolated from Islet-1+ neonatal cardiovascular progenitor cells. **(D)** Corresponding visualization of YAP1 protein. **(E)** Analysis of phosphorylated YAP1/YAP1 ratio. **(F)** Quantification of Phosphorylated YAP1 and ß-Actin protein levels in the adult CPC following exosome treatment. Fold changes are shown as the mean ± SEM. All samples were run in quadruplicate and normalized to a housekeeping gene, Actin.

YAP1-associated Transcripts Involved in Proliferation are Elevated in Adult Cardiac Progenitor Cell Clones Following a 72-hour Treatment with Islet-1+ Neonatal Cardiac Progenitor Cell-derived Exosomes

YAP1 is able to interact with ß-catenin in order to regulate levels of SOX2, a transcript that supports cardiomyocyte proliferation (Lopez-Hernandez et al., 2021). Following a 72-hour co-culture of neonatal cardiac progenitor cell-derived exosomes with adult cardiac progenitor cells, we observed a significant increase in transcripts encoding SOX2 (2.45 ± 0.40 FC, **** $p<0.0001$) as shown in Figure 4A, 4B. Intranuclear YAP1 translocation also upregulates CCNA2, a transcript present during the G_2/M phase transition of the cell cycle (Z. Lin et al., 2015a; Zanconato et al., 2015). CCNA2 transcripts were elevated (1.24 ± 0.12 FC, * $p=0.0462$) in the adult CPC following a 72-hour treatment with exosomes as

shown in Figure 4C. DNA-binding transcription factors such as ERBB4 interact with YAP1 and are associated with proliferation (Haskins et al., 2014a). The YAP1-ERBB4 complex regulates organ and tissue growth (M.-K. Kim et al., 2018). ERBB4 transcripts were found to be significantly increased (7.27 ± 5.24 FC, * p=0.0230) in the adult CPC after exosome treatment, as shown in Figure 4D. Collectively, we identified several transcripts that are associated with proliferation, and which were induced in adult cardiovascular progenitor cell clones following a short-term exposure to neonatal cardiovascular progenitor cell-derived exosomes. We next addressed the influence of exosomes on the AKT signaling pathway due to the well-documented crosstalk with AKT and YAP1 in proliferative, pro-survival networks (Z. Lin et al., 2015b).

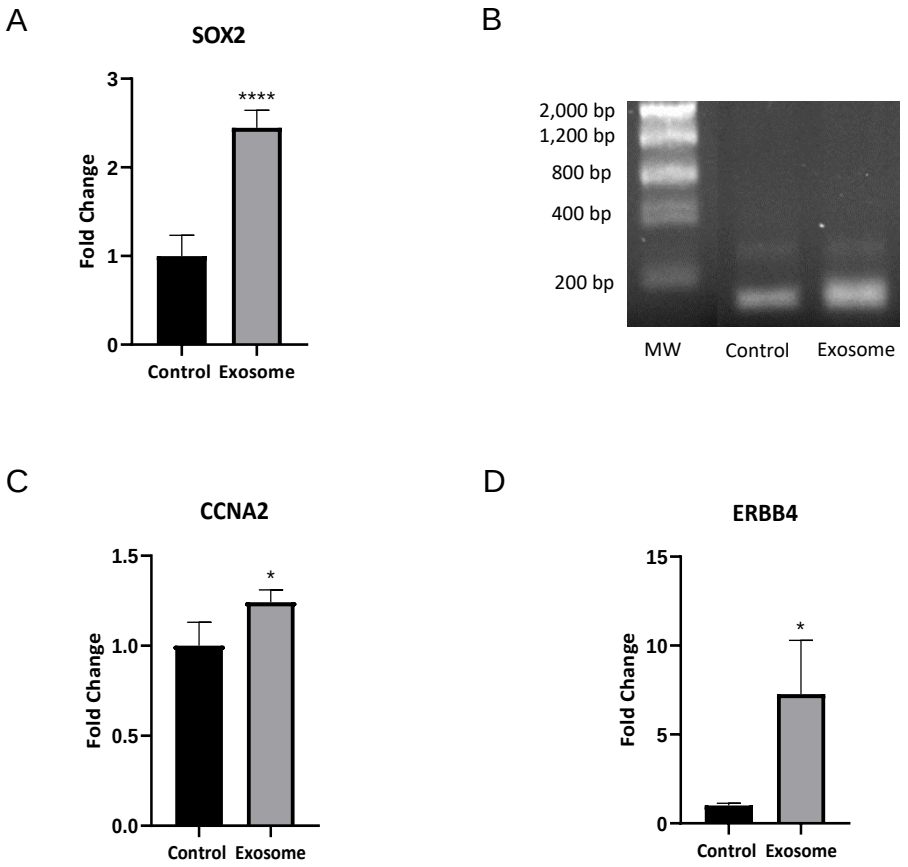


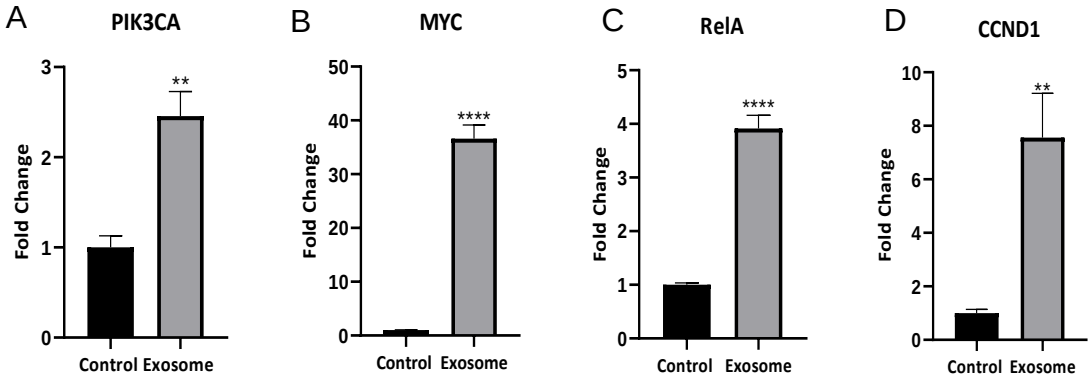
Figure 4. YAP1-associated Transcripts Involved in Proliferation are Elevated in Adult Cardiac Progenitor Cell Clones After Exosome Treatment

(A) SOX2 is significantly elevated in adult CPCs following a 72-hour treatment with exosomes isolated from Islet-1+ neonatal cardiovascular progenitor cells as shown by RT-qPCR. **(B)** Gel electrophoresis of the RT-qPCR product SOX2, amplifies products of the correct size. **(C)** CCNA2 which is present during the G_2/M phase transition of the cell cycle, is significantly elevated in the adult CPC following a 72-hour treatment with exosomes isolated from Islet-1+ neonatal cardiovascular progenitor cells as confirmed by RT-qPCR. **(D)** ERBB4 transcript levels are significantly elevated in the adult CPC following a 72-hour treatment with exosomes as shown by RT-qPCR. Samples were run in quadruplicate **(A)** or triplicate **(C, D).** All transcripts were normalized to the housekeeping gene, Actin. Fold changes are shown as the mean $\pm$ SEM.

Transcripts Associated with the AKT Signaling Pathway are Elevated in the Adult CPC Following a 72-hour Treatment with Exosomes Derived from Neonatal Islet-1+ Cardiac Progenitor Cells

The AKT signaling pathway can modulate cellular functions important in regeneration such as; cell cycle progression, cellular proliferation, motility, differentiation, angiogenesis, metabolism and cellular survival (Akinleye et al., 2013; Long et al., 2021; Song et al., 2018; Xue & Hemmings, 2013). Transcripts associated with the AKT signaling pathway in adult CPCs were elevated as demonstrated by RT-qPCR after treatment with exosomes derived from neonatal cardiovascular progenitors for 72-hours. Since expression of YAP1 activation results in elevated expression of genes in the AKT signaling pathway such as *Pik3ca* and *Pik3cb* (Z. Lin et al., 2015b), we examined PIK3CA transcripts. PIK3CA is able to activate AKT via the PI3K-AKT signaling pathway (P. Liu et al., 2009). We observed a significant increase in PIK3CA expression (2.46 ± 0.47 FC, ** p=0.0011) in adult CPCs after exosome treatment, as shown in Figure 5A, 5E. Increased transcription of pro-proliferative genes in this pathway, such as MYC, occurs through downstream targeting of PI3K-AKT and intranuclear YAP1 transcriptional activation (Abeyrathna & Su, 2015; Borreguero-Muñoz et al., 2019a; Monroe et al., 2019). MYC positively regulates G1/S phase cell cycle progression by regulation of cyclin E and CDK2 (Santoni-Rugiu et al., 2000) and accordingly, MYC transcripts were elevated (36.59 ± 4.39 FC, **** p<0.0001) in adult CPCs after co-culture with neonatal CPC exosomes as shown in Figure 5B,

5E. AKT stimulates the transcription factor NF-κβ; a heterodimer composed of subunits p50 and RelA/p65 (Uzdensky et al., 2013). RelA/p65 can induce YAP1 nuclear localization and inhibit its degradation (T. Zhao et al., 2022). Both NF-κβ and YAP1 influence cell-cycle control and proliferation by regulating the transcription of CCND1 (Hinz et al., 1999; Mizuno et al., 2012; Uzdensky et al., 2013; Yamaguchi & Taouk, 2020). As a result of exosome treatment, RelA (3.92 ± 0.42 FC, **** p<0.0001) and CCND1 (7.56 ± 2.86 FC, ** p=0.0024) transcripts were significantly increased in adult CPCs as shown in Figure 5C, 5D, 5E.



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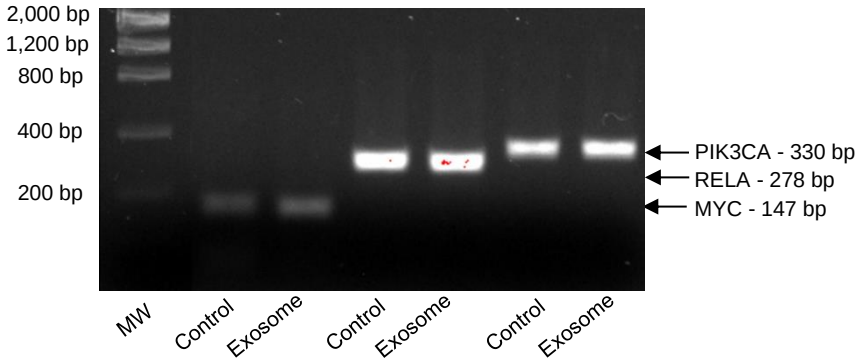

Figure 5. Transcripts Associated with the AKT Signaling Pathway are Elevated in Adult CPCs after Neonatal Exosome Treatment

Quantitative RT-PCR data demonstrating transcripts associated with the AKT signaling pathway such as **(A)** PIK3CA **(B)** MYC **(C)** RelA and **(D)** CCND1 which were found to be significantly elevated in adult CPCs following a 72-hour co-culture with exosomes isolated from Islet-1+ neonatal cardiovascular progenitor cells. **(E)** Gel electrophoresis of the RT-qPCR products MYC, RelA, and PIK3CA showing amplified products of the correct sizes (left to right): 147bp, 278bp, and 330bp. All samples were run in triplicate and all transcripts were normalized to the housekeeping gene, Actin. Fold changes are shown as the mean ± SEM.

Proposed Signaling Pathway by Which Adult Cardiac Progenitor Cells are Induced to Proliferate after Neonatal CPC-derived Exosome Treatment

It is known that exosomes can mediate downstream signaling events through ligand-receptor interactions or through fusion and release of exosome contents into the cytosol. This subsequently alters physiological properties of the recipient cell (Adamiak & Sahoo, 2018; Gurung et al., 2021c; Urbanelli et al., 2013). In the context of influencing cellular proliferation, PI3K-AKT and Hippo signaling interaction has been illustrated mainly through receptor tyrosine kinases (Azad et al., 2020). Upon stimulation of these ligand-receptor interactions, the phosphorylation of PIK3CA/p110α, PIK3CB/p110β, or p110δ/PIK3CD and a p85 regulatory subunit will drive further activation of downstream signaling in the PI3K-AKT signaling pathway (Gangoda et al., 2015; Mazloumi Gavgani et al., 2018). Activation of PI3K-AKT signaling was observed in Figure 5A, 5E by an increase in PIK3CA expression (2.46 ± 0.47 FC, ** p=0.0011). AKT phosphorylates MST1/2 and the downstream effects can lead to nuclear YAP1 activity (Borreguero-Muñoz et al., 2019b; Ibar & Irvine, 2020; D.

Kim et al., 2010). YAP1 activation leads to transcription of downstream targets such as CTGF (Emmanouilidi & Falasca, 2017; R. Fan et al., 2013). We found an increase in CTGF transcript levels (1.62 ± 0.33 FC, *** p<0.0004) in adult CPCs after co-culture of neonatal CPC-derived exosomes, as shown in Figure 6B. CTGF has been shown to provoke cell cycle activity by significantly stimulating DNA synthesis in neonatal mammals (O'Meara et al., 2015a).

Active AKT can also phosphorylate the IKK complex which leads to the enhancement of NF-κβ transcriptional activity (Bai et al., 2009; Dan et al., 2008). NF-κβ contains a family of transcriptional factors that function in inflammation, immunity, cellular proliferation, differentiation and survival (Oeckinghaus & Ghosh, 2009). Some of these targets include MYC and CCND1 (Hariri et al., 2013). AKT phosphorylates CREB which leads to transcription of downstream genes involved in cellular proliferation and survival such as BCL-2, CCND1 and Cyclin A (CCNA2) (H. Wang et al., 2018a). CREB can also activate expression of YAP1 through AKT signaling (Yu et al., 2019), which was found to be elevated in adult CPCs (2.16 ± 0.329 FC, **** p<0.0001) after neonatal CPC exosome treatment, as shown in Figure 6A. Both CREB and YAP1 can facilitate transcription of downstream genes involved in cellular cycle such as CCND1 and CCNA2 (Rozengurt et al., 2018; H. Wang et al., 2018b). However, YAP1 has only been shown to transcriptionally induce SOX2 transcripts by occupying the promoter region of this gene (Bora-Singhal et al., 2015).

ERBB4 transcripts which are present in cardiovascular progenitor cell-derived exosomes can further activate YAP1 nuclear transcriptional activity (Haskins et al., 2014b). Collectively, ERBB4, AKT-associated, and YAP1 transcripts which are elevated by exosome treatment, lead to proliferation and cell cycle activity as shown in Figure 6C.

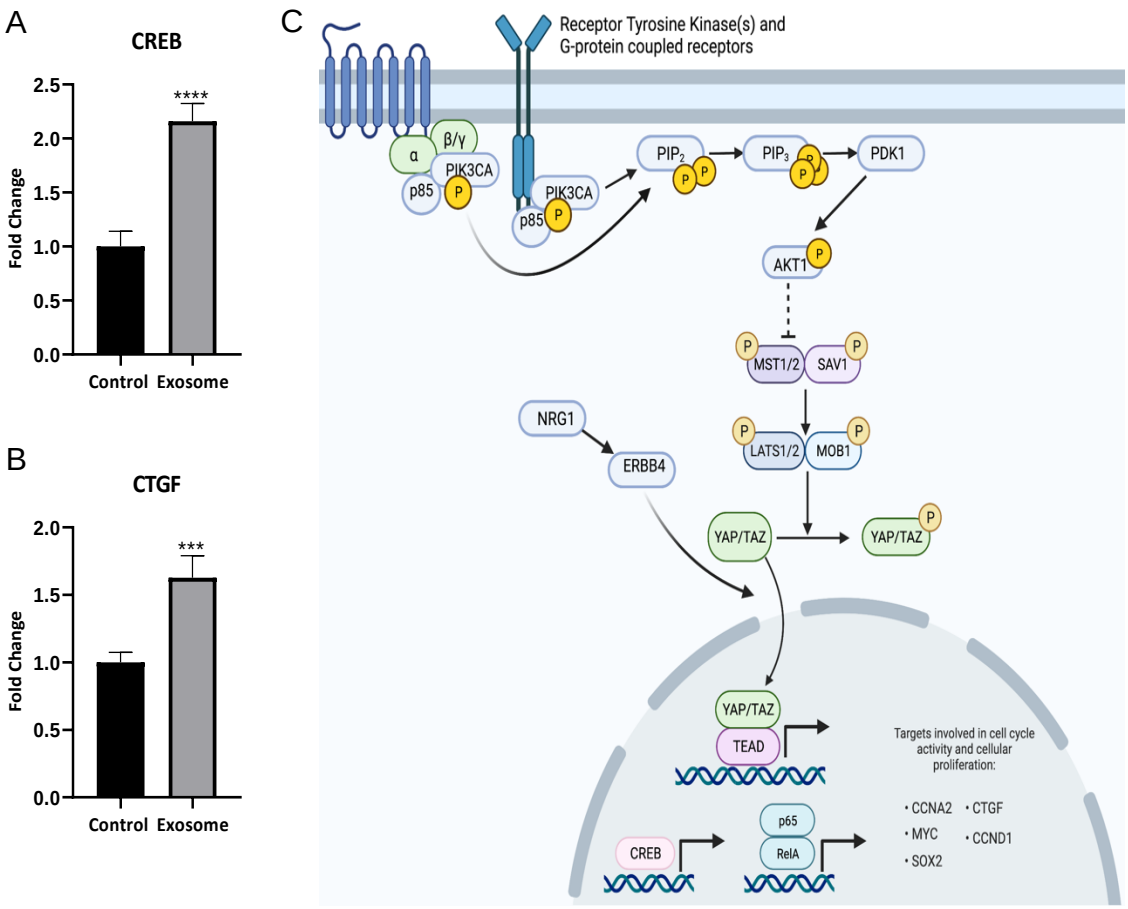

Figure 6. A Proposed Signaling Mechanism in Adult Cardiovascular Progenitors After Neonatal Cardiac Progenitor Cell-derived Exosome Treatment

(A) CREB transcripts were found to be significantly elevated in adult CPCs after neonatal CPC exosome treatment **(B)** CTGF, a known downstream transcript of the Hippo signaling pathway, was increased in adult CPCs after co-culture with exosomes isolated from neonatal cardiovascular progenitors, as shown by RT-qPCR **(C)** Proposed signaling mechanism in adult CPCs upon co-culture of exosomes isolated from neonatal Islet-1+ cardiac progenitor cells. Image created in BioRender.com. All samples were run in quadruplicate and normalized to the housekeeping gene, Actin. Fold changes are shown as the mean $\pm$ SEM.

CHAPTER FOUR

DISCUSSION

Over the past couple of years, stem and progenitor cells and their exosomes have gained noteworthy recognition as a therapeutic approach for the purpose of cardiac regeneration and repair (reviewed in Mehanna et al., 2022). A robust regenerative response has been observed in the neonatal heart after being able to withstand apical resection in order to sufficiently stimulate functionality to the damaged myocardium (Lam & Sadek, 2018; Porrello et al., 2011). This is due to the ability of the neonatal heart to proliferate in the early stages of the neonatal period. This unique feature subsequently deteriorates following birth (Bongiovanni et al., 2021). It is also well known that the adult mammalian heart has a limited capacity for repair given that the resident cardiomyocytes have exited the cellular cycle (Bongiovanni et al., 2021). Therefore, a therapeutic strategy to allow for proliferation to occur and for resident myocytes to enter the cell cycle in the adult myocardium after injury is necessary for regeneration.

To our knowledge, current therapies with the use of stem-derived exosomes to attenuate the injured heart by cardiac regeneration are still being extensively studied in pre-clinical phases. This is due to need for standard exosome isolation and characterization protocols, optimal yield, targeted delivery, and the importance of identifying the optimal parent cell (Kwon, 2022; J. Zhang et

al., 2021). None of these studies have utilized exosomes derived from human neonatal cardiovascular progenitors with early-stage markers such as Isl1+.

In this study, we have co-cultured neonatal Islet-1+ CPC-derived exosomes with adult cardiovascular progenitors to test the hypothesis that adult cardiovascular progenitors will have improved transcriptomic changes associated with improved regenerative capabilities *in vitro* when co-cultured with exosomes isolated from neonatal CPCs. The findings of this study present the benefits of the secretome of early-stage Islet-1+ neonatal cardiovascular progenitors when applied to adult cardiovascular progenitors.

We have identified an optimal method of exosome isolation and we have characterized the size of the isolated particles which we found to be similar to well-characterized exosomes (Yi et al., 2020b). We found that 5 – 6 $x10^{10}$ exosomes were needed in order to identify a functional increase in cellular proliferation, similar to the concentration noted as necessary to achieve beneficial functional outcomes in other settings (El Harane et al., 2018b; J. Sun et al., 2020; Yao et al., 2021b).

Within the neonatal CPC exosome content we have reported several miRNAs that contribute to signaling pathways that are important in cardiac development and regeneration (Hesse et al., 2018; H. Li et al., 2021; Ozhan & Weidinger, 2015; Wadugu & Kühn, 2012). Differentially expressed miRNAs in neonatal CPC-derived exosomes which were identified by DIANA-miRPath v3.0 bioinformatics software included: cell cycle, Wnt signaling, ERBB signaling, and

Notch signaling. This data suggests that the use of exosomes derived from neonatal cardiovascular progenitors could potentially impact these pathways to drive regenerative-like processes. We also reported several other miRNAs and transcripts in the neonatal CPC exosome content that significantly contributed to pathways which connect to the Hippo signaling pathway such as TGF-beta, Focal adhesion, CREB signaling, ERBB signaling, and G-protein coupled (GPCR) signaling (Han, 2019; Haskins et al., 2014a; Luo & Yu, 2019; Morice et al., 2020; 2018).

Upon close investigation into the transcriptomics data associated with GPCR signaling, we found that YAP1 activation was predicted in the pathway, leading to downstream transcriptional activity that results in proliferation of cells and AKT-mediated activation of NF-κβ. We also found that intranuclear YAP1 was predicted by transcriptomic analysis of the exosome content in neonatal CPCs. *In vitro* validation experiments demonstrate the activation of transcripts encoding YAP1 and RelA (subunit of NF-κβ) in adult CPCs after exosome treatment in Figures 3A, 3B, 5C, 5E. This shows that the transcripts found within the exosomes of neonatal cardiovascular progenitors provide a translational function when applied to adult CPCs *in vitro*.

We identified several miRNAs in the neonatal cardiovascular progenitors that are exclusively different from the adult CPC secretome such as miR-31 and miR-221. These miRNAs are all capable of targeting LATS and subsequently activating YAP1 transcriptional activity (Aguennouz et al., 2020; Mitamura et al.,

2014), which is necessary for targeting cardiac repair. Overexpression of miR-31 in cells has been shown to lead to YAP1 translocation, where it promoted the transcription of CCND1 (Mitamura et al., 2014). This finding is comparable with the increased transcription of CCND1 and YAP1 protein in this study. More importantly, miR-221 has been shown to be important in a miRNA cocktail which significantly improved functional recovery by regulating cell survival and apoptosis in a murine myocardial infarction model (S. Hu et al., 2011).

We also reported that miRNA-221 can function in promoting proliferation by modulation of cell-cycle genes, an essential process that could be harnessed for cardiac repair and therapy (Fornari et al., 2008). We observed another important miRNA, miR-133a1 which was found to be expressed in neonatal CPC exosomes. This miRNA has been shown to enhance the protective capacity of cardiovascular progenitors in a myocardial infarction model by targeting genes related to cell death, hypertrophy, fibrosis, and apoptosis all which lead to *in vitro* contractile functional effects in the heart (Izarra et al., 2014).

During myocardial infarction, extensive cardiomyocyte death, inflammation and fibrosis can occur which leads to fibrotic scar tissue replacement (Thomas & Grisanti, 2020). We have identified miRNAs in the neonatal exosome content which target fibrosis and inflammation. We identified miR-133a1 in our neonatal CPC exosome content which has been shown to have anti-fibrotic properties when applied by transfection *in vivo* (Y. Chen et al., 2017). We have also identified a miRNA from the miR-181 family, miR-181a, expressed in neonatal

CPC exosomes (X. Sun et al., 2014). This miRNA has been previously shown to inhibit the proinflammatory transcription factor c-FOS to repress pro-inflammatory responses upon transfection (C. Wu et al., 2012). Our data suggests that the secretome of the Islet-1+ neonatal cardiovascular progenitors has beneficial miRNAs and transcripts that promote cell cycle activity, can play a part in heart repair processes, and can contribute to pathways associated with proliferation such as Hippo signaling which leads to YAP1 activation. This content contributes to their functional benefits when applied to the adult CPC in vitro.

YAP1 has been shown to induce cardiomyocytes to re-enter the cellular cycle and proliferate. In addition, activation of YAP1 can trigger surrounding cells through secreted factors to promote tissue repair (Gong et al., 2021b; Mugahid et al., 2020b). YAP1 can facilitate the regenerative potential of the adult heart (Xin et al., 2013a). These findings support the concept that YAP1 is a critical component of cardiac repair mechanisms. We have demonstrated that the addition of early-stage Islet-1+ neonatal progenitor-derived exosomes significantly altered transcript and protein levels of YAP1, which suggests YAP1 activation and nuclear translocation (Fig 3 A, B, C, and D). We also observe a significant increase in the ratio of phosphorylated YAP1 relative to the non-phosphorylated YAP1 protein (Fig 3F). Some studies have suggested that YAP1 phosphorylated at Serine 127 is still able to accumulate into the nucleus (Piccolo et al., 2014b). Our model identifies Serine 127 phosphorylation of YAP1 during protein visualization by the Protein Simple Western blot system. Consistent with

this finding, we still observed activation of downstream transcripts such as CTGF (B. Zhao et al., 2008), in the adult CPC after neonatal cardiovascular progenitor cell-derived exosome treatment. CTGF triggers cell cycle activity in neonatal mammals, cellular proliferation, cellular migration and adhesion, angiogenesis and early wound healing and repair (O'Meara et al., 2015b; Shome et al., 2020). ERBB4 was identified in neonatal exosomes by transcriptomics and similarly activates YAP1 to promote downstream transcriptional activity (Haskins et al., 2014c). CREB was also elevated in adult treated cells and can induce YAP1 transcriptional function (J. Wang et al., 2013). YAP1 is a powerful driver of organ growth and progenitor proliferation (Barry et al., 2013).

Modification of adult CPCs by exosome treatment resulting in elevated YAP1 transcription could improve cardiovascular repair outcomes. YAP1 administration in a myocardial infarction model stimulates adult cardiomyocyte proliferation via IGF-1 and AKT signaling (Xin et al., 2013b). Our findings show that transcripts involved with the PI3K-AKT signaling pathway are activated in our model and include elevated levels of PI3KCA, c-MYC, RELA, and CCND1.

CCND1 plays a critical role in promoting G1-S phase, while CCNA2 has a role in G1-S and G2-M transitions of the cellular cycle (Q. Wang et al., 2018; Xing et al., 2021). CCNA2 is a key player in the process of DNA replication since it combines with CDK2 in order to progress to mitosis (Kanakkanthara et al., 2016). Neonatal cardiovascular progenitor cell-derived exosomes contain transcripts that promote the cell cycle and the cell cycle control of chromosomal

replication. The exosomes activate cell cycle transcripts CCND1 and CCNA2 after co-culture with adult CPCs. This data supports the hypothesis that neonatal CPC-derived exosomes can alter the transcriptome of the adult cardiac progenitor cells and achieve a proliferative-like state by activating cell cycle transcripts. We have shown that several components contribute to YAP1 activation in the adult CPC by neonatal cardiovascular progenitor cell exosome treatment after 72 hours and this leads to activation of transcripts involved in cellular cycle and proliferation which are necessary for cardiac repair.

Conclusion

Our findings provide evidence that neonatal cardiovascular progenitor cell-derived exosomes contain valuable molecules that enhance the proliferative ability of adult cardiovascular progenitor cells upon co-culture *in vitro*. The neonatal cardiovascular progenitor cells contain miRNAs and transcripts that induce YAP1 transcription and activate the cell cycle in adult CPCs. This was assessed by observation of 1) Elevated levels of YAP1 transcript and protein levels 2) Elevated levels of transcripts important in PI3K-AKT signaling such as PI3K and RelA and some that have function in cell cycle-progression and proliferation such as c-MYC and CCND1 3) Elevation of transcripts involved G1-S and G2-M progression in the cellular cycle such as CCNA2. YAP1 activation and transcriptional alteration in adult CPCs by neonatal cardiovascular progenitor cell-derived exosomes could have clinical benefit in cardiovascular repair through

potential activation of signaling in the recipient and potentially in the surrounding cell types when applied as a cell-based treatment.

REFERENCES

Abeyrathna, P., & Su, Y. (2015). The Critical Role of Akt in Cardiovascular Function. *Vascular Pharmacology*, *74*, 38–48. https://doi.org/10.1016/j.vph.2015.05.008

Adamiak, M., Cheng, G., Bobis-Wozowicz, S., Zhao, L., Kedracka-Krok, S., Samanta, A., Karnas, E., Xuan, Y.-T., Skupien-Rabian, B., Chen, X., Jankowska, U., Girgis, M., Sekula, M., Davani, A., Lasota, S., Vincent, R. J., Sarna, M., Newell, K. L., Wang, O.-L., … Zuba-Surma, E. K. (2018). Induced Pluripotent Stem Cell (iPSC)–Derived Extracellular Vesicles Are Safer and More Effective for Cardiac Repair Than iPSCs. *Circulation Research*, *122*(2), 296–309. https://doi.org/10.1161/CIRCRESAHA.117.311769

Adamiak, M., & Sahoo, S. (2018). Exosomes in Myocardial Repair: Advances and Challenges in the Development of Next-Generation Therapeutics. *Molecular Therapy*, *26*(7), 1635–1643. https://doi.org/10.1016/j.ymthe.2018.04.024

Aguennouz, M., Polito, F., Visalli, M., Vita, G., Raffa, G., Oteri, R., Ghazi, B., Scalia, G., Angileri, F. F., Barresi, V., Caffo, M., Cardali, S., Conti, A., Macaione, V., Bartolotta, M., Giorgio, R. D., & Germanò, A. (2020). MicroRNA-10 and -221 modulate differential expression of Hippo signaling pathway in human astroglial tumors. *Cancer Treatment and Research Communications*, *24*, 100203. https://doi.org/10.1016/j.ctarc.2020.100203

Aharonov, A., Shakked, A., Umansky, K. B., Savidor, A., Genzelinakh, A., Kain, D., Lendengolts, D., Revach, O.-Y., Morikawa, Y., Dong, J., Levin, Y., Geiger, B., Martin, J. F., & Tzahor, E. (2020). ERBB2 drives YAP activation and EMT-like

processes during cardiac regeneration. *Nature Cell Biology*, *22*(11), 1346–1356. https://doi.org/10.1038/s41556-020-00588-4

Ahuja, P., Sdek, P., & Maclellan, W. R. (2007). Cardiac Myocyte Cell Cycle Control in Development, Disease and Regeneration. *Physiological Reviews*, *87*(2), 521–544. https://doi.org/10.1152/physrev.00032.2006

Akinleye, A., Avvaru, P., Furqan, M., Song, Y., & Liu, D. (2013). Phosphatidylinositol 3-kinase (PI3K) inhibitors as cancer therapeutics. *Journal of Hematology & Oncology*, *6*, 88. https://doi.org/10.1186/1756-8722-6-88

Andriolo, G., Provasi, E., Lo Cicero, V., Brambilla, A., Soncin, S., Torre, T., Milano, G., Biemmi, V., Vassalli, G., Turchetto, L., Barile, L., & Radrizzani, M. (2018). Exosomes From Human Cardiac Progenitor Cells for Therapeutic Applications: Development of a GMP-Grade Manufacturing Method. *Frontiers in Physiology*, *9*. https://www.frontiersin.org/article/10.3389/fphys.2018.01169

Artap, S., Manderfield, L. J., Smith, C. L., Poleshko, A., Aghajanian, H., See, K., Li, L., Jain, R., & Epstein, J. A. (2018). Endocardial Hippo signaling regulates myocardial growth and cardiogenesis. *Developmental Biology*, *440*(1), 22–30. https://doi.org/10.1016/j.ydbio.2018.04.026

Atluri, P., Miller, J. S., Emery, R. J., Hung, G., Trubelja, A., Cohen, J. E., Lloyd, K., Han, J., Gaffey, A. C., MacArthur, J. W., Chen, C. S., & Woo, Y. J. (2014). Tissue-engineered, hydrogel-based endothelial progenitor cell therapy robustly revascularizes ischemic myocardium and preserves ventricular function. *The*

Journal of Thoracic and Cardiovascular Surgery, *148*(3), 1090–1098. https://doi.org/10.1016/j.jtcvs.2014.06.038

Austin, A. F., Compton, L. A., Love, J. D., Brown, C. B., & Barnett, J. V. (2008). Primary and immortalized mouse epicardial cells undergo differentiation in response to TGFβ. *Developmental Dynamics*, *237*(2), 366–376. https://doi.org/10.1002/dvdy.21421

Ayala-Mar, S., Donoso-Quezada, J., Gallo-Villanueva, R. C., Perez-Gonzalez, V. H., & González-Valdez, J. (2019). Recent advances and challenges in the recovery and purification of cellular exosomes. *ELECTROPHORESIS*, *40*(23–24), 3036–3049. https://doi.org/10.1002/elps.201800526

Azad, T., Rezaei, R., Surendran, A., Singaravelu, R., Boulton, S., Dave, J., Bell, J. C., & Ilkow, C. S. (2020). Hippo Signaling Pathway as a Central Mediator of Receptors Tyrosine Kinases (RTKs) in Tumorigenesis. *Cancers*, *12*(8), 2042. https://doi.org/10.3390/cancers12082042

Bai, D., Ueno, L., & Vogt, P. K. (2009). Akt-mediated regulation of NFκB and the essentialness of NFκB for the oncogenicity of PI3K and Akt. *International Journal of Cancer. Journal International Du Cancer*, *125*(12), 2863–2870. https://doi.org/10.1002/ijc.24748

Baio, J., Martinez, A. F., Silva, I., Hoehn, C. V., Countryman, S., Bailey, L., Hasaniya, N., Pecaut, M. J., & Kearns-Jonker, M. (2018). Cardiovascular progenitor cells cultured aboard the International Space Station exhibit altered developmental

and functional properties. *Npj Microgravity*, *4*(1), 13. https://doi.org/10.1038/s41526-018-0048-x

Balbi, C., Milano, G., Fertig, T. E., Lazzarini, E., Bolis, S., Taniyama, Y., Sanada, F., Di Silvestre, D., Mauri, P., Gherghiceanu, M., Lüscher, T. F., Barile, L., & Vassalli, G. (2021). An exosomal-carried short periostin isoform induces cardiomyocyte proliferation. *Theranostics*, *11*(12), 5634–5649. https://doi.org/10.7150/thno.57243

Balbi, C., & Vassalli, G. (2020). Exosomes: Beyond stem cells for cardiac protection and repair. *STEM CELLS*, *38*(11), 1387–1399. https://doi.org/10.1002/stem.3261

Barile, L., Cervio, E., Lionetti, V., Milano, G., Ciullo, A., Biemmi, V., Bolis, S., Altomare, C., Matteucci, M., Di Silvestre, D., Brambilla, F., Fertig, T. E., Torre, T., Demertzis, S., Mauri, P., Moccetti, T., & Vassalli, G. (2018). Cardioprotection by cardiac progenitor cell-secreted exosomes: Role of pregnancy-associated plasma protein-A. *Cardiovascular Research*, *114*(7), 992–1005. https://doi.org/10.1093/cvr/cvy055

Barile, L., Lionetti, V., Cervio, E., Matteucci, M., Gherghiceanu, M., Popescu, L. M., Torre, T., Siclari, F., Moccetti, T., & Vassalli, G. (2014). Extracellular vesicles from human cardiac progenitor cells inhibit cardiomyocyte apoptosis and improve cardiac function after myocardial infarction. *Cardiovascular Research*, *103*(4), 530–541. https://doi.org/10.1093/cvr/cvu167

Barry, E. R., Morikawa, T., Butler, B. L., Shrestha, K., de la Rosa, R., Yan, K. S., Fuchs, C. S., Magness, S. T., Smits, R., Ogino, S., Kuo, C. J., & Camargo, F. D. (2013).

Restriction of intestinal stem cell expansion and the regenerative response by YAP. *Nature*, *493*(7430), 106–110. https://doi.org/10.1038/nature11693

Bartulos, O., Zhuang, Z. W., Huang, Y., Mikush, N., Suh, C., Bregasi, A., Wang, L., Chang, W., Krause, D. S., Young, L. H., Pober, J. S., & Qyang, Y. (n.d.). ISL1 cardiovascular progenitor cells for cardiac repair after myocardial infarction. *JCI Insight*, *1*(10), e80920. https://doi.org/10.1172/jci.insight.80920

Bellio, M. A., Kanashiro-Takeuchi, R. M., Takeuchi, L., Kulandavelu, S., Lee, Y.-S., Balkan, W., Young, K. C., Hare, J. M., & Khan, A. (2022). Systemic delivery of large-scale manufactured Wharton's Jelly mesenchymal stem cell-derived extracellular vesicles improves cardiac function after myocardial infarction. *The Journal of Cardiovascular Aging*, *2*(1), 9. https://doi.org/10.20517/jca.2021.21

Bergmann, O., Bhardwaj, R. D., Bernard, S., Zdunek, S., Barnabé-Heider, F., Walsh, S., Zupicich, J., Alkass, K., Buchholz, B. A., Druid, H., Jovinge, S., & Frisén, J. (2009). Evidence for Cardiomyocyte Renewal in Humans. *Science*, *324*(5923), 98–102. https://doi.org/10.1126/science.1164680

Bergmann, O., Zdunek, S., Felker, A., Salehpour, M., Alkass, K., Bernard, S., Sjostrom, S. L., Szewczykowska, M., Jackowska, T., dos Remedios, C., Malm, T., Andrä, M., Jashari, R., Nyengaard, J. R., Possnert, G., Jovinge, S., Druid, H., & Frisén, J. (2015). Dynamics of Cell Generation and Turnover in the Human Heart. *Cell*, *161*(7), 1566–1575. https://doi.org/10.1016/j.cell.2015.05.026

Bertokova, A., Svecova, N., Kozics, K., Gabelova, A., Vikartovska, A., Jane, E., Hires, M., Bertok, T., & Tkac, J. (2022). Exosomes from prostate cancer cell lines:

Isolation optimisation and characterisation. *Biomedicine & Pharmacotherapy*, *151*, 113093. https://doi.org/10.1016/j.biopha.2022.113093

Bojmar, L., Kim, H. S., Tobias, G. C., Pelissier Vatter, F. A., Lucotti, S., Gyan, K. E., Kenific, C. M., Wan, Z., Kim, K.-A., Kim, D., Hernandez, J., Pascual, V., Heaton, T. E., La Quaglia, M. P., Kelsen, D., Trippett, T. M., Jones, D. R., Jarnagin, W. R., Matei, I. R., … Lyden, D. (2021). Extracellular vesicle and particle isolation from human and murine cell lines, tissues, and bodily fluids. *STAR Protocols*, *2*(1), 100225. https://doi.org/10.1016/j.xpro.2020.100225

Bollini, S., Smart, N., & Riley, P. R. (2011). Resident cardiac progenitor cells: At the heart of regeneration. *Journal of Molecular and Cellular Cardiology*, *50*(2), 296–303. https://doi.org/10.1016/j.yjmcc.2010.07.006

Bongiovanni, C., Sacchi, F., Da Pra, S., Pantano, E., Miano, C., Morelli, M. B., & D'Uva, G. (2021). Reawakening the Intrinsic Cardiac Regenerative Potential: Molecular Strategies to Boost Dedifferentiation and Proliferation of Endogenous Cardiomyocytes. *Frontiers in Cardiovascular Medicine*, *8*. https://www.frontiersin.org/article/10.3389/fcvm.2021.750604

Bora-Singhal, N., Nguyen, J., Schaal, C., Perumal, D., Singh, S., Coppola, D., & Chellappan, S. (2015). YAP1 Regulates OCT4 Activity and SOX2 Expression to Facilitate Self-Renewal and Vascular Mimicry of Stem-Like Cells. *Stem Cells (Dayton, Ohio)*, *33*(6), 1705–1718. https://doi.org/10.1002/stem.1993

Borreguero-Muñoz, N., Fletcher, G. C., Aguilar-Aragon, M., Elbediwy, A., Vincent-Mistiaen, Z. I., & Thompson, B. J. (2019). The Hippo pathway integrates PI3K–

Akt signals with mechanical and polarity cues to control tissue growth. *PLOS Biology*, *17*(10), e3000509. https://doi.org/10.1371/journal.pbio.3000509

Brennan, K., Martin, K., FitzGerald, S. P., O'Sullivan, J., Wu, Y., Blanco, A., Richardson, C., & Mc Gee, M. M. (2020). A comparison of methods for the isolation and separation of extracellular vesicles from protein and lipid particles in human serum. *Scientific Reports*, *10*(1), 1039. https://doi.org/10.1038/s41598-020-57497-7

Butreddy, A., Kommineni, N., & Dudhipala, N. (2021). Exosomes as Naturally Occurring Vehicles for Delivery of Biopharmaceuticals: Insights from Drug Delivery to Clinical Perspectives. *Nanomaterials*, *11*(6), 1481. https://doi.org/10.3390/nano11061481

Cai, C.-L., Liang, X., Shi, Y., Chu, P.-H., Pfaff, S. L., Chen, J., & Evans, S. (2003). Isl1 Identifies a Cardiac Progenitor Population that Proliferates Prior to Differentiation and Contributes a Majority of Cells to the Heart. *Developmental Cell*, *5*(6), 877–889. https://doi.org/10.1016/S1534-5807(03)00363-0

Camberos, V., Baio, J., Mandujano, A., Martinez, A. F., Bailey, L., Hasaniya, N., & Kearns-Jonker, M. (2021). The Impact of Spaceflight and Microgravity on the Human Islet-1+ Cardiovascular Progenitor Cell Transcriptome. *International Journal of Molecular Sciences*, *22*(7), 3577. https://doi.org/10.3390/ijms22073577

Camps-Vilaró, A., Delgado-Jiménez, J. F., Farré, N., Tizón-Marcos, H., Álvarez-García, J., Cinca, J., Dégano, I. R., & Marrugat, J. (2020). Estimated Population

Prevalence of Heart Failure with Reduced Ejection Fraction in Spain, According to DAPA-HF Study Criteria. *Journal of Clinical Medicine*, *9*(7), 2089. https://doi.org/10.3390/jcm9072089

Cao, N., Liang, H., & Yang, H.-T. (2015). Generation, expansion, and differentiation of cardiovascular progenitor cells from human pluripotent stem cells. *Methods in Molecular Biology (Clifton, N.J.)*, *1212*, 113–125. https://doi.org/10.1007/7651_2014_119

Chen, J., Li, P., Zhang, T., Xu, Z., Huang, X., Wang, R., & Du, L. (2022). Review on Strategies and Technologies for Exosome Isolation and Purification. *Frontiers in Bioengineering and Biotechnology*, *9*. https://www.frontiersin.org/article/10.3389/fbioe.2021.811971

Chen, L., Wang, Y., Pan, Y., Zhang, L., Shen, C., Qin, G., Ashraf, M., Weintraub, N., Ma, G., & Tang, Y. (2013). Cardiac progenitor-derived exosomes protect ischemic myocardium from acute ischemia/reperfusion injury. *Biochemical and Biophysical Research Communications*, *431*(3), 566–571. https://doi.org/10.1016/j.bbrc.2013.01.015

Chen, X., Li, Y., Luo, J., & Hou, N. (2020). Molecular Mechanism of Hippo–YAP1/TAZ Pathway in Heart Development, Disease, and Regeneration. *Frontiers in Physiology*, *11*. https://www.frontiersin.org/article/10.3389/fphys.2020.00389

Chen, Y., Zhao, Y., Chen, W., Xie, L., Zhao, Z.-A., Yang, J., Chen, Y., Lei, W., & Shen, Z. (2017). MicroRNA-133 overexpression promotes the therapeutic efficacy of

mesenchymal stem cells on acute myocardial infarction. *Stem Cell Research & Therapy*, *8*(1), 268. https://doi.org/10.1186/s13287-017-0722-z

Cheng, G., Zhu, D., Huang, K., & Caranasos, T. G. (2022). Minimally invasive delivery of a hydrogel-based exosome patch to prevent heart failure. *Journal of Molecular and Cellular Cardiology*, *169*, 113–121. https://doi.org/10.1016/j.yjmcc.2022.04.020

Chernyavskaya, Y., Ebert, A. M., Milligan, E., & Garrity, D. M. (2012). Voltage-gated calcium channel CACNB2 (β2.1) protein is required in the heart for control of cell proliferation and heart tube integrity. *Developmental Dynamics*, *241*(4), 648–662. https://doi.org/10.1002/dvdy.23746

Chia, D. J. (2014). Minireview: Mechanisms of Growth Hormone-Mediated Gene Regulation. *Molecular Endocrinology*, *28*(7), 1012–1025. https://doi.org/10.1210/me.2014-1099

Choi, J., Lee, S., Clement, K., Mallard, W., Tagliazucchi, G. M., Lim, H., Choi, I. Y., Ferrari, F., Tsankov, A., Pop, R., Lee, G., Rinn, J., Meissner, A., Park, P. J., & Hochedlinger, K. (2015). A comparison of genetically matched cell lines reveals the equivalence of human iPSCs and ESCs. *Nature Biotechnology*, *33*(11), 1173–1181. https://doi.org/10.1038/nbt.3388

Chung, J. J., Han, J., Wang, L. L., Arisi, M. F., Zaman, S., Gordon, J., Li, E., Kim, S. T., Tran, Z., Chen, C. W., Gaffey, A. C., Burdick, J. A., & Atluri, P. (2020). Delayed delivery of endothelial progenitor cell-derived extracellular vesicles via shear thinning gel improves postinfarct hemodynamics. *The Journal of Thoracic and*

Cardiovascular Surgery, *159*(5), 1825-1835.e2. https://doi.org/10.1016/j.jtcvs.2019.06.017

Ciullo, A., Biemmi, V., Milano, G., Bolis, S., Cervio, E., Fertig, E. T., Gherghiceanu, M., Moccetti, T., Camici, G. G., Vassalli, G., & Barile, L. (2019). Exosomal Expression of CXCR4 Targets Cardioprotective Vesicles to Myocardial Infarction and Improves Outcome after Systemic Administration. *International Journal of Molecular Sciences*, *20*(3), 468. https://doi.org/10.3390/ijms20030468

Comfort, N., Cai, K., Bloomquist, T. R., Strait, M. D., Ferrante, A. W., & Baccarelli, A. A. (2021). Nanoparticle Tracking Analysis for the Quantification and Size Determination of Extracellular Vesicles. *Journal of Visualized Experiments : JoVE*, *169*, 10.3791/62447. https://doi.org/10.3791/62447

Coughlan, C., Bruce, K., Burgy, O., Boyd, T. D., Michel, C. R., Garcia-Perez, J. E., Adame, V., Anton, P., Bettcher, B. M., Chial, H. J., Koenigshoff, M., Hsieh, E. W. Y., Graner, M., & Potter, H. (2020). Exosome Isolation by Ultracentrifugation and Precipitation: A Comparison of Techniques for Downstream Analyses. *Current Protocols in Cell Biology*, *88*(1), e110. https://doi.org/10.1002/cpcb.110

Dagenais, G. R., Leong, D. P., Rangarajan, S., Lanas, F., Lopez-Jaramillo, P., Gupta, R., Diaz, R., Avezum, A., Oliveira, G. B. F., Wielgosz, A., Parambath, S. R., Mony, P., Alhabib, K. F., Temizhan, A., Ismail, N., Chifamba, J., Yeates, K., Khatib, R., Rahman, O., … Yusuf, S. (2020). Variations in common diseases, hospital admissions, and deaths in middle-aged adults in 21 countries from five

continents (PURE): A prospective cohort study. *The Lancet*, *395*(10226), 785–794. https://doi.org/10.1016/S0140-6736(19)32007-0

Dan, H. C., Cooper, M. J., Cogswell, P. C., Duncan, J. A., Ting, J. P.-Y., & Baldwin, A. S. (2008). Akt-dependent regulation of NF-κB is controlled by mTOR and Raptor in association with IKK. *Genes & Development*, *22*(11), 1490–1500. https://doi.org/10.1101/gad.1662308

Danac, J. M. C., Uy, A. G. G., & Garcia, R. L. (2021). Exosomal microRNAs in colorectal cancer: Overcoming barriers of the metastatic cascade (Review). *International Journal of Molecular Medicine*, *47*(6), 1–16. https://doi.org/10.3892/ijmm.2021.4945

Daneshgar, N., Rabinovitch, P. S., & Dai, D.-F. (2021). TOR Signaling Pathway in Cardiac Aging and Heart Failure. *Biomolecules*, *11*(2), 168. https://doi.org/10.3390/biom11020168

de Couto, G., Gallet, R., Cambier, L., Jaghatspanyan, E., Makkar, N., Dawkins, J. F., Berman, B. P., & Marbán, E. (2017). Exosomal microRNA transfer into macrophages mediates cellular postconditioning de Couto: Exosomal RNA transfer modulates macrophages. *Circulation*, *136*(2), 200–214. https://doi.org/10.1161/CIRCULATIONAHA.116.024590

Deinsberger, J., Reisinger, D., & Weber, B. (2020). Global trends in clinical trials involving pluripotent stem cells: A systematic multi-database analysis. *Npj Regenerative Medicine*, *5*(1), 1–13. https://doi.org/10.1038/s41536-020-00100-4

del Campo, C. V., Liaw, N. Y., Gunadasa-Rohling, M., Matthaei, M., Braga, L., Kennedy, T., Salinas, G., Voigt, N., Giacca, M., Zimmermann, W.-H., & Riley, P. R. (2021). Regenerative potential of epicardium-derived extracellular vesicles mediated by conserved miRNA transfer. *Cardiovascular Research*, *118*(2), 597–611. https://doi.org/10.1093/cvr/cvab054

Deleidi, M., Hargus, G., Hallett, P., Osborn, T., & Isacson, O. (2011). Development of Histocompatible Primate-Induced Pluripotent Stem Cells for Neural Transplantation. *Stem Cells*, *29*(7), 1052–1063. https://doi.org/10.1002/stem.662

Devine, W. P., Wythe, J. D., George, M., Koshiba-Takeuchi, K., & Bruneau, B. G. (n.d.). Early patterning and specification of cardiac progenitors in gastrulating mesoderm. *ELife*, *3*, e03848. https://doi.org/10.7554/eLife.03848

Dougherty, J. A., Patel, N., Kumar, N., Rao, S. G., Angelos, M. G., Singh, H., Cai, C., & Khan, M. (2020). Human Cardiac Progenitor Cells Enhance Exosome Release and Promote Angiogenesis Under Physoxia. *Frontiers in Cell and Developmental Biology*, *8*, 130. https://doi.org/10.3389/fcell.2020.00130

Drowley, L., Koonce, C., Peel, S., Jonebring, A., Plowright, A. T., Kattman, S. J., Andersson, H., Anson, B., Swanson, B. J., Wang, Q.-D., & Brolen, G. (2016). Human Induced Pluripotent Stem Cell-Derived Cardiac Progenitor Cells in Phenotypic Screening: A Transforming Growth Factor-β Type 1 Receptor Kinase Inhibitor Induces Efficient Cardiac Differentiation. *Stem Cells Translational Medicine*, *5*(2), 164–174. https://doi.org/10.5966/sctm.2015-0114

El Harane, N., Kervadec, A., Bellamy, V., Pidial, L., Neametalla, H. J., Perier, M.-C., Lima Correa, B., Thiébault, L., Cagnard, N., Duché, A., Brunaud, C., Lemitre, M., Gauthier, J., Bourdillon, A. T., Renault, M. P., Hovhannisyan, Y., Paiva, S., Colas, A. R., Agbulut, O., … Renault, N. K. E. (2018). Acellular therapeutic approach for heart failure: In vitro production of extracellular vesicles from human cardiovascular progenitors. *European Heart Journal*, *39*(20), 1835–1847. https://doi.org/10.1093/eurheartj/ehy012

Emmanouilidi, A., & Falasca, M. (2017). Targeting PDK1 for Chemosensitization of Cancer Cells. *Cancers*, *9*(10), 140. https://doi.org/10.3390/cancers9100140

Fan, D., Wu, H., Pan, K., Peng, H., & Wu, R. (2021). Regenerating Damaged Myocardium: A Review of Stem-Cell Therapies for Heart Failure. *Cells*, *10*(11), 3125. https://doi.org/10.3390/cells10113125

Fan, R., Kim, N.-G., & Gumbiner, B. M. (2013). Regulation of Hippo pathway by mitogenic growth factors via phosphoinositide 3-kinase and phosphoinositide-dependent kinase-1. *Proceedings of the National Academy of Sciences*, *110*(7), 2569–2574. https://doi.org/10.1073/pnas.1216462110

Fingar, D. C., Richardson, C. J., Tee, A. R., Cheatham, L., Tsou, C., & Blenis, J. (2004). MTOR Controls Cell Cycle Progression through Its Cell Growth Effectors S6K1 and 4E-BP1/Eukaryotic Translation Initiation Factor 4E. *Molecular and Cellular Biology*, *24*(1), 200–216. https://doi.org/10.1128/MCB.24.1.200-216.2004

Flinn, M. A., Link, B. A., & O'Meara, C. C. (2020). Upstream regulation of the Hippo-Yap pathway in cardiomyocyte regeneration. *Seminars in Cell & Developmental Biology*, *100*, 11–19. https://doi.org/10.1016/j.semcdb.2019.09.004

Foo, K. S., Lehtinen, M. L., Leung, C. Y., Lian, X., Xu, J., Keung, W., Geng, L., Kolstad, T. R. S., Thams, S., Wong, A. O., Wong, N., Bylund, K., Zhou, C., He, X., Jin, S.-B., Clarke, J., Lendahl, U., Li, R. A., Louch, W. E., & Chien, K. R. (2018). Human ISL1+ Ventricular Progenitors Self-Assemble into an In Vivo Functional Heart Patch and Preserve Cardiac Function Post Infarction. *Molecular Therapy*, *26*(7), 1644–1659. https://doi.org/10.1016/j.ymthe.2018.02.012

Fornari, F., Gramantieri, L., Ferracin, M., Veronese, A., Sabbioni, S., Calin, G. A., Grazi, G. L., Giovannini, C., Croce, C. M., Bolondi, L., & Negrini, M. (2008). MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. *Oncogene*, *27*(43), 5651–5661. https://doi.org/10.1038/onc.2008.178

Fuentes, T. I., Appleby, N., Tsay, E., Martinez, J. J., Bailey, L., Hasaniya, N., & Kearns-Jonker, M. (2013). Human Neonatal Cardiovascular Progenitors: Unlocking the Secret to Regenerative Ability. *PLOS ONE*, *8*(10), e77464. https://doi.org/10.1371/journal.pone.0077464

Gallet, R., Dawkins, J., Valle, J., Simsolo, E., de Couto, G., Middleton, R., Tseliou, E., Luthringer, D., Kreke, M., Smith, R. R., Marbán, L., Ghaleh, B., & Marbán, E. (2017). Exosomes secreted by cardiosphere-derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic

porcine myocardial infarction. *European Heart Journal*, *38*(3), 201–211. https://doi.org/10.1093/eurheartj/ehw240

Gangoda, L., Boukouris, S., Liem, M., Kalra, H., & Mathivanan, S. (2015). Extracellular vesicles including exosomes are mediators of signal transduction: Are they protective or pathogenic? *Proteomics*, *15*(0), 260–271. https://doi.org/10.1002/pmic.201400234

Gao, R., Liang, X., Cheedipudi, S., Cordero, J., Jiang, X., Zhang, Q., Caputo, L., Günther, S., Kuenne, C., Ren, Y., Bhattacharya, S., Yuan, X., Barreto, G., Chen, Y., Braun, T., Evans, S. M., Sun, Y., & Dobreva, G. (2019). Pioneering function of Isl1 in the epigenetic control of cardiomyocyte cell fate. *Cell Research*, *29*(6), 486–501. https://doi.org/10.1038/s41422-019-0168-1

Gardiner, C., Ferreira, Y. J., Dragovic, R. A., Redman, C. W. G., & Sargent, I. L. (2013). Extracellular vesicle sizing and enumeration by nanoparticle tracking analysis. *Journal of Extracellular Vesicles*, *2*, 10.3402/jev.v2i0.19671. https://doi.org/10.3402/jev.v2i0.19671

Garza, M. A., Wason, E. A., & Zhang, J. Q. (2015). Cardiac remodeling and physical training post myocardial infarction. *World Journal of Cardiology*, *7*(2), 52–64. https://doi.org/10.4330/wjc.v7.i2.52

Ghazizadeh, Z., Fattahi, F., Mirzaei, M., Bayersaikhan, D., Lee, J., Chae, S., Hwang, D., Byun, K., Tabar, M. S., Taleahmad, S., Mirshahvaladi, S., Shabani, P., Fonoudi, H., Haynes, P. A., Baharvand, H., Aghdami, N., Evans, T., Lee, B., & Salekdeh, G. H. (2018). Prospective Isolation of ISL1+ Cardiac Progenitors from Human

ESCs for Myocardial Infarction Therapy. *Stem Cell Reports*, *10*(3), 848–859. https://doi.org/10.1016/j.stemcr.2018.01.037

Gong, R., Jiang, Z., Zagidullin, N., Liu, T., & Cai, B. (2021). Regulation of cardiomyocyte fate plasticity: A key strategy for cardiac regeneration. *Signal Transduction and Targeted Therapy*, *6*(1), 1–11. https://doi.org/10.1038/s41392-020-00413-2

Gray, W. D., French, K. M., Ghosh-Choudhary, S., Maxwell, J. T., Brown, M. E., Platt, M. O., Searles, C. D., & Davis, M. E. (2015). Identification of Therapeutic Covariant microRNA Clusters in Hypoxia Treated Cardiac Progenitor Cell Exosomes using Systems Biology. *Circulation Research*, *116*(2), 255–263. https://doi.org/10.1161/CIRCRESAHA.116.304360

Günthel, M., Barnett, P., & Christoffels, V. M. (2018). Development, Proliferation, and Growth of the Mammalian Heart. *Molecular Therapy*, *26*(7), 1599–1609. https://doi.org/10.1016/j.ymthe.2018.05.022

Gurung, S., Perocheau, D., Touramanidou, L., & Baruteau, J. (2021). The exosome journey: From biogenesis to uptake and intracellular signalling. *Cell Communication and Signaling*, *19*(1), 47. https://doi.org/10.1186/s12964-021-00730-1

Han, Y. (2019). Analysis of the role of the Hippo pathway in cancer. *Journal of Translational Medicine*, *17*(1), 116. https://doi.org/10.1186/s12967-019-1869-4

Hariri, F., Arguello, M., Volpon, L., Culjkovic-Kraljacic, B., Nielsen, T. H., Hiscott, J., Mann, K. K., & Borden, K. L. B. (2013). The eukaryotic translation initiation factor eIF4E is a direct transcriptional target of NF-κB and is aberrantly regulated in

Acute Myeloid Leukemia. *Leukemia*, *27*(10), 2047–2055. https://doi.org/10.1038/leu.2013.73

Haskins, J. W., Nguyen, D. X., & Stern, D. F. (2014). Neuregulin 1-activated ERBB4 interacts with YAP to induce Hippo pathway targetgenes and promote cell migration. *Science Signaling*, *7*(355), ra116. https://doi.org/10.1126/scisignal.2005770

Hentze, H., Soong, P. L., Wang, S. T., Phillips, B. W., Putti, T. C., & Dunn, N. R. (2009). Teratoma formation by human embryonic stem cells: Evaluation of essential parameters for future safety studies. *Stem Cell Research*, *2*(3), 198–210. https://doi.org/10.1016/j.scr.2009.02.002

Hesse, M., Welz, A., & Fleischmann, B. K. (2018). Heart regeneration and the cardiomyocyte cell cycle. *Pflugers Archiv*, *470*(2), 241–248. https://doi.org/10.1007/s00424-017-2061-4

Hinz, M., Krappmann, D., Eichten, A., Heder, A., Scheidereit, C., & Strauss, M. (1999). NF-κB Function in Growth Control: Regulation of Cyclin D1 Expression and G0/G1-to-S-Phase Transition. *Molecular and Cellular Biology*, *19*(4), 2690–2698.

Hirai, K., Ousaka, D., Fukushima, Y., Kondo, M., Eitoku, T., Shigemitsu, Y., Hara, M., Baba, K., Iwasaki, T., Kasahara, S., Ohtsuki, S., & Oh, H. (2020). Cardiosphere-derived exosomal microRNAs for myocardial repair in pediatric dilated cardiomyopathy. *Science Translational Medicine*, *12*(573), eabb3336. https://doi.org/10.1126/scitranslmed.abb3336

Hou, Y., & Li, C. (2018). Stem/Progenitor Cells and Their Therapeutic Application in Cardiovascular Disease. *Frontiers in Cell and Developmental Biology*, *6*, 139. https://doi.org/10.3389/fcell.2018.00139

Hu, H. T., Nishimura, T., & Suetsugu, S. (2021). Ultracentrifugal separation, characterization, and functional study of extracellular vesicles derived from serum-free cell culture. *STAR Protocols*, *2*(3), 100625. https://doi.org/10.1016/j.xpro.2021.100625

Hu, S., Huang, M., Nguyen, P. K., Gong, Y., Li, Z., Jia, F., Lan, F., Liu, J., Nag, D., Robbins, R. C., & Wu, J. C. (2011). Novel MicroRNA Pro-survival Cocktail for Improving Engraftment and Function of Cardiac Progenitor Cell Transplantation. *Circulation*, *124*(11 Suppl), S27–S34. https://doi.org/10.1161/CIRCULATIONAHA.111.017954

Ibar, C., & Irvine, K. D. (2020). Integration of Hippo-YAP Signaling with Metabolism. *Developmental Cell*, *54*(2), 256–267. https://doi.org/10.1016/j.devcel.2020.06.025

Izarra, A., Moscoso, I., Levent, E., Cañón, S., Cerrada, I., Díez-Juan, A., Blanca, V., Núñez-Gil, I.-J., Valiente, I., Ruíz-Sauri, A., Sepúlveda, P., Tiburcy, M., Zimmermann, W.-H., & Bernad, A. (2014). MiR-133a Enhances the Protective Capacity of Cardiac Progenitors Cells after Myocardial Infarction. *Stem Cell Reports*, *3*(6), 1029–1042. https://doi.org/10.1016/j.stemcr.2014.10.010

Jayaraman, S., Gnanasampanthapandian, D., Rajasingh, J., & Palaniyandi, K. (2021). Stem Cell-Derived Exosomes Potential Therapeutic Roles in Cardiovascular

Diseases. *Frontiers in Cardiovascular Medicine*, *8*. https://www.frontiersin.org/article/10.3389/fcvm.2021.723236

Jia, L., Gu, W., Zhang, Y., Jiang, B., Qiao, X., & Wen, Y. (2018). Activated Yes-Associated Protein Accelerates Cell Cycle, Inhibits Apoptosis, and Delays Senescence in Human Periodontal Ligament Stem Cells. *International Journal of Medical Sciences*, *15*(11), 1241–1250. https://doi.org/10.7150/ijms.25115

Kalluri, R., & LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. *Science (New York, N.Y.)*, *367*(6478), eaau6977. https://doi.org/10.1126/science.aau6977

Kanakkanthara, A., Jeganathan, K. B., Limzerwala, J. F., Baker, D. J., Hamada, M., Nam, H.-J., van Deursen, W. H., Hamada, N., Naylor, R. M., Becker, N. A., Davies, B. A., van Ree, J. H., Mer, G., Shapiro, V. S., Maher, L. J., Katzmann, D. J., & van Deursen, J. M. (2016). Cyclin A2 is an RNA binding protein that controls Mre11 mRNA translation. *Science (New York, N.Y.)*, *353*(6307), 1549–1552. https://doi.org/10.1126/science.aaf7463

Kang, T., Jones, T. M., Naddell, C., Bacanamwo, M., Calvert, J. W., Thompson, W. E., Bond, V. C., Chen, Y. E., & Liu, D. (2016). Adipose-Derived Stem Cells Induce Angiogenesis via Microvesicle Transport of miRNA-31. *Stem Cells Translational Medicine*, *5*(4), 440–450. https://doi.org/10.5966/sctm.2015-0177

Kasai-Brunswick, T. H., Carvalho, A. B., & Campos de Carvalho, A. C. (2021). Stem cell therapies in cardiac diseases: Current status and future possibilities. *World*

Journal of Stem Cells, *13*(9), 1231–1247. https://doi.org/10.4252/wjsc.v13.i9.1231

Kehat, I., Kenyagin-Karsenti, D., Snir, M., Segev, H., Amit, M., Gepstein, A., Livne, E., Binah, O., Itskovitz-Eldor, J., & Gepstein, L. (2001). Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *Journal of Clinical Investigation*, *108*(3), 407–414.

Kiermayer, S., Biondi, R. M., Imig, J., Plotz, G., Haupenthal, J., Zeuzem, S., & Piiper, A. (2005). Epac Activation Converts cAMP from a Proliferative into a Differentiation Signal in PC12 Cells. *Molecular Biology of the Cell*, *16*(12), 5639–5648. https://doi.org/10.1091/mbc.e05-05-0432

Kim, D., Shu, S., Coppola, M. D., Kaneko, S., Yuan, Z., & Cheng, J. Q. (2010). Regulation of Proapoptotic Mammalian ste20–Like Kinase MST2 by the IGF1-Akt Pathway. *PLOS ONE*, *5*(3), e9616. https://doi.org/10.1371/journal.pone.0009616

Kim, M.-K., Jang, J.-W., & Bae, S.-C. (2018). DNA binding partners of YAP/TAZ. *BMB Reports*, *51*(3), 126–133. https://doi.org/10.5483/BMBRep.2018.51.3.015

Kwon, I. S. K. & K. (2022). Potential application of biomimetic exosomes in cardiovascular disease: Focused on ischemic heart disease. *BMB Reports*, *55*(1), 30–38. https://doi.org/10.5483/BMBRep.2022.55.1.161

Lai, T.-C., Lee, T.-L., Chang, Y.-C., Chen, Y.-C., Lin, S.-R., Lin, S.-W., Pu, C.-M., Tsai, J.-S., & Chen, Y.-L. (2020). MicroRNA-221/222 Mediates ADSC-Exosome-Induced Cardioprotection Against Ischemia/Reperfusion by Targeting PUMA and

ETS-1. *Frontiers in Cell and Developmental Biology*, *8*. https://www.frontiersin.org/article/10.3389/fcell.2020.569150

Lam, N. T., & Sadek, H. A. (2018). Neonatal Heart Regeneration. *Circulation*, *138*(4), 412–423. https://doi.org/10.1161/CIRCULATIONAHA.118.033648

Laugwitz, K.-L., Moretti, A., Caron, L., Nakano, A., & Chien, K. R. (2008). Islet1 cardiovascular progenitors: A single source for heart lineages? *Development*, *135*(2), 193–205. https://doi.org/10.1242/dev.001883

Le, T., & Chong, J. (2016). Cardiac progenitor cells for heart repair. *Cell Death Discovery*, *2*, 16052. https://doi.org/10.1038/cddiscovery.2016.52

Lee, A. S., Tang, C., Cao, F., Xie, X., van der Bogt, K., Hwang, A., Connolly, A. J., Robbins, R. C., & Wu, J. C. (2009). Effects of cell number on teratoma formation by human embryonic stem cells. *Cell Cycle (Georgetown, Tex.)*, *8*(16), 2608–2612.

Lee, M.-O., Moon, S. H., Jeong, H.-C., Yi, J.-Y., Lee, T.-H., Shim, S. H., Rhee, Y.-H., Lee, S.-H., Oh, S.-J., Lee, M.-Y., Han, M.-J., Cho, Y. S., Chung, H.-M., Kim, K.-S., & Cha, H.-J. (2013). Inhibition of pluripotent stem cell-derived teratoma formation by small molecules. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(35), E3281–E3290. https://doi.org/10.1073/pnas.1303669110

Li, H., Chang, C., Li, X., & Zhang, R. (2021). The roles and activation of endocardial Notch signaling in heart regeneration. *Cell Regeneration*, *10*(1), 3. https://doi.org/10.1186/s13619-020-00060-6

Li, T.-S., Cheng, K., Lee, S.-T., Matsushita, S., Davis, D., Malliaras, K., Zhang, Y., Matsushita, N., Smith, R. R., & Marbán, E. (2010). Cardiospheres Recapitulate a Niche-Like Microenvironment Rich in Stemness and Cell-Matrix Interactions, Rationalizing Their Enhanced Functional Potency for Myocardial Repair. *Stem Cells*, *28*(11), 2088–2098. https://doi.org/10.1002/stem.532

Li, Y., Tian, S., Lei, I., Liu, L., Ma, P., & Wang, Z. (2017). Transplantation of multipotent Isl1+ cardiac progenitor cells preserves infarcted heart function in mice. *American Journal of Translational Research*, *9*(3), 1530–1542.

Liang, B., He, X., Zhao, Y.-X., Zhang, X.-X., & Gu, N. (2020). Advances in Exosomes Derived from Different Cell Sources and Cardiovascular Diseases. *BioMed Research International*, *2020*, e7298687. https://doi.org/10.1155/2020/7298687

Liang, G., & Zhang, Y. (2013). Genetic and Epigenetic Variations in iPSCs: Potential Causes and Implications for Application. *Cell Stem Cell*, *13*(2), 149–159. https://doi.org/10.1016/j.stem.2013.07.001

Lima Correa, B., El Harane, N., Desgres, M., Perotto, M., Alayrac, P., Guillas, C., Pidial, L., Bellamy, V., Baron, E., Autret, G., Kamaleswaran, K., Pezzana, C., Perier, M.-C., Vilar, J., Alberdi, A., Brisson, A., Renault, N., Gnecchi, M., Silvestre, J.-S., & Menasché, P. (2021). Extracellular vesicles fail to trigger the generation of new cardiomyocytes in chronically infarcted hearts. *Theranostics*, *11*(20), 10114–10124. https://doi.org/10.7150/thno.62304

Lima Correa, B., El Harane, N., Gomez, I., Rachid Hocine, H., Vilar, J., Desgres, M., Bellamy, V., Keirththana, K., Guillas, C., Perotto, M., Pidial, L., Alayrac, P., Tran,

T., Tan, S., Hamada, T., Charron, D., Brisson, A., Renault, N. K., Al-Daccak, R., … Silvestre, J.-S. (2021). Extracellular vesicles from human cardiovascular progenitors trigger a reparative immune response in infarcted hearts. *Cardiovascular Research*, *117*(1), 292–307. https://doi.org/10.1093/cvr/cvaa028

Lin, Y.-N., Mesquita, T., Sanchez, L., Chen, Y.-H., Liu, W., Li, C., Rogers, R., Wang, Y., Li, X., Wu, D., Zhang, R., Ibrahim, A., Marbán, E., & Cingolani, E. (2021). Extracellular vesicles from immortalized cardiosphere-derived cells attenuate arrhythmogenic cardiomyopathy in desmoglein-2 mutant mice. *European Heart Journal*, *42*(35), 3558–3571. https://doi.org/10.1093/eurheartj/ehab419

Lin, Z., & Pu, W. T. (2014). Harnessing Hippo in the heart: Hippo/Yap signaling and applications to heart regeneration and rejuvenation. *Stem Cell Research*, *13*(3, Part B), 571–581. https://doi.org/10.1016/j.scr.2014.04.010

Lin, Z., Zhou, P., von Gise, A., Gu, F., Ma, Q., Chen, J., Guo, H., van Gorp, P. R. R., Wang, D.-Z., & Pu, W. T. (2015). Pi3kcb Links Hippo-YAP and PI3K-AKT Signaling Pathways to Promote Cardiomyocyte Proliferation and Survival. *Circulation Research*, *116*(1), 35–45. https://doi.org/10.1161/CIRCRESAHA.115.304457

Liu, B., Lee, B. W., Nakanishi, K., Villasante, A., Williamson, R., Metz, J., Kim, J., Kanai, M., Bi, L., Brown, K., Di Paolo, G., Homma, S., Sims, P. A., Topkara, V. K., & Vunjak-Novakovic, G. (2018). Cardiac recovery via extended cell-free delivery of extracellular vesicles secreted by cardiomyocytes derived from induced

pluripotent stem cells. *Nature Biomedical Engineering*, *2*(5), 293–303. https://doi.org/10.1038/s41551-018-0229-7

Liu, P., Cheng, H., Roberts, T. M., & Zhao, J. J. (2009). Targeting the phosphoinositide 3-kinase (PI3K) pathway in cancer. *Nature Reviews. Drug Discovery*, *8*(8), 627–644. https://doi.org/10.1038/nrd2926

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Livshits, M. A., Khomyakova, E., Evtushenko, E. G., Lazarev, V. N., Kulemin, N. A., Semina, S. E., Generozov, E. V., & Govorun, V. M. (2015). Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol. *Scientific Reports*, *5*(1), 17319. https://doi.org/10.1038/srep17319

Long, H.-Z., Cheng, Y., Zhou, Z.-W., Luo, H.-Y., Wen, D.-D., & Gao, L.-C. (2021). PI3K/AKT Signal Pathway: A Target of Natural Products in the Prevention and Treatment of Alzheimer's Disease and Parkinson's Disease. *Frontiers in Pharmacology*, *12*. https://www.frontiersin.org/article/10.3389/fphar.2021.648636

Lopez-Hernandez, A., Sberna, S., & Campaner, S. (2021). Emerging Principles in the Transcriptional Control by YAP and TAZ. *Cancers*, *13*(16), 4242. https://doi.org/10.3390/cancers13164242

Ludwig, N., Whiteside, T. L., & Reichert, T. E. (2019). Challenges in Exosome Isolation and Analysis in Health and Disease. *International Journal of Molecular Sciences*, *20*(19), 4684. https://doi.org/10.3390/ijms20194684

Luo, J., & Yu, F.-X. (2019). GPCR-Hippo Signaling in Cancer. *Cells*, *8*(5), 426. https://doi.org/10.3390/cells8050426

Luther, K. M., Haar, L., McGuinness, M., Wang, Y., Lynch IV, T. L., Phan, A., Song, Y., Shen, Z., Gardner, G., Kuffel, G., Ren, X., Zilliox, M. J., & Jones, W. K. (2018). Exosomal miR-21a-5p mediates cardioprotection by mesenchymal stem cells. *Journal of Molecular and Cellular Cardiology*, *119*, 125–137. https://doi.org/10.1016/j.yjmcc.2018.04.012

Maring, J. A., Lodder, K., Mol, E., Verhage, V., Wiesmeijer, K. C., Dingenouts, C. K. E., Moerkamp, A. T., Deddens, J. C., Vader, P., Smits, A. M., Sluijter, J. P. G., & Goumans, M.-J. (2019). Cardiac Progenitor Cell–Derived Extracellular Vesicles Reduce Infarct Size and Associate with Increased Cardiovascular Cell Proliferation. *Journal of Cardiovascular Translational Research*, *12*(1), 5–17. https://doi.org/10.1007/s12265-018-9842-9

Mauretti, A., Spaans, S., Bax, N. A. M., Sahlgren, C., & Bouten, C. V. C. (2017). Cardiac Progenitor Cells and the Interplay with Their Microenvironment. *Stem Cells International*, *2017*, 1–20. https://doi.org/10.1155/2017/7471582

Mazloumi Gavgani, F., Smith Arnesen, V., Jacobsen, R. G., Krakstad, C., Hoivik, E. A., & Lewis, A. E. (2018). Class I Phosphoinositide 3-Kinase PIK3CA/p110α and PIK3CB/p110β Isoforms in Endometrial Cancer. *International Journal of Molecular Sciences*, *19*(12), 3931. https://doi.org/10.3390/ijms19123931

Mehanna, R. A., Essawy, M. M., Barkat, M. A., Awaad, A. K., Thabet, E. H., Hamed, H. A., Elkafrawy, H., Khalil, N. A., Sallam, A., Kholief, M. A., Ibrahim, S. S., &

Mourad, G. M. (2022). Cardiac stem cells: Current knowledge and future prospects. *World Journal of Stem Cells*, *14*(1), 1–40. https://doi.org/10.4252/wjsc.v14.i1.1

Meng, Z., Moroishi, T., & Guan, K.-L. (2016). Mechanisms of Hippo pathway regulation. *Genes & Development*, *30*(1), 1–17. https://doi.org/10.1101/gad.274027.115

Mia, M. M., & Singh, M. K. (2019). The Hippo Signaling Pathway in Cardiac Development and Diseases. *Frontiers in Cell and Developmental Biology*, *7*, 211. https://doi.org/10.3389/fcell.2019.00211

Milano, G., Biemmi, V., Lazzarini, E., Balbi, C., Ciullo, A., Bolis, S., Ameri, P., Di Silvestre, D., Mauri, P., Barile, L., & Vassalli, G. (2020). Intravenous administration of cardiac progenitor cell-derived exosomes protects against doxorubicin/trastuzumab-induced cardiac toxicity. *Cardiovascular Research*, *116*(2), 383–392. https://doi.org/10.1093/cvr/cvz108

Mitamura, T., Watari, H., Wang, L., Kanno, H., Kitagawa, M., Hassan, M. K., Kimura, T., Tanino, M., Nishihara, H., Tanaka, S., & Sakuragi, N. (2014). MicroRNA 31 functions as an endometrial cancer oncogene by suppressing Hippo tumor suppressor pathway. *Molecular Cancer*, *13*(1), 97. https://doi.org/10.1186/1476-4598-13-97

Mitton, B., Chae, H.-D., Hsu, K., Dutta, R., Aldana-Masangkay, G., Ferrari, R., Davis, K., Tiu, B. C., Kaul, A., Lacayo, N., Dahl, G., Xie, F., Li, B. X., Breese, M. R., Landaw, E. M., Nolan, G., Pellegrini, M., Romanov, S., Xiao, X., & Sakamoto, K. M. (2016). Small molecule inhibition of cAMP response element binding protein

in human acute myeloid leukemia cells. *Leukemia*, *30*(12), 2302–2311. https://doi.org/10.1038/leu.2016.139

Mizuno, T., Murakami, H., Fujii, M., Ishiguro, F., Tanaka, I., Kondo, Y., Akatsuka, S., Toyokuni, S., Yokoi, K., Osada, H., & Sekido, Y. (2012). YAP induces malignant mesothelioma cell proliferation by upregulating transcription of cell cycle-promoting genes. *Oncogene*, *31*(49), 5117–5122. https://doi.org/10.1038/onc.2012.5

Mol, E. A., Goumans, M. J., & Sluijter, J. P. G. (2017). Cardiac Progenitor-Cell Derived Exosomes as Cell-Free Therapeutic for Cardiac Repair. In J. Xiao & S. Cretoiu (Eds.), *Exosomes in Cardiovascular Diseases: Biomarkers, Pathological and Therapeutic Effects* (pp. 207–219). Springer. https://doi.org/10.1007/978-981-10-4397-0_14

Monroe, T. O., Hill, M. C., Morikawa, Y., Leach, J. P., Heallen, T., Cao, S., Krijger, P. H. L., de Laat, W., Wehrens, X. H. T., Rodney, G. G., & Martin, J. F. (2019). YAP Partially Reprograms Chromatin Accessibility to Directly Induce Adult Cardiogenesis In Vivo. *Developmental Cell*, *48*(6), 765-779.e7. https://doi.org/10.1016/j.devcel.2019.01.017

Moradi, S., Mahdizadeh, H., Šarić, T., Kim, J., Harati, J., Shahsavarani, H., Greber, B., & Moore, J. B. (2019). Research and therapy with induced pluripotent stem cells (iPSCs): Social, legal, and ethical considerations. *Stem Cell Research & Therapy*, *10*(1), 341. https://doi.org/10.1186/s13287-019-1455-y

Morice, S., Danieau, G., Rédini, F., Brounais-Le-Royer, B., & Verrecchia, F. (2020). Hippo/YAP Signaling Pathway: A Promising Therapeutic Target in Bone Paediatric Cancers? *Cancers*, *12*(3), 645. https://doi.org/10.3390/cancers12030645

Mugahid, D., Kalocsay, M., Liu, X., Gruver, J. S., Peshkin, L., & Kirschner, M. W. (2020). YAP regulates cell size and growth dynamics via non-cell autonomous mediators. *ELife*, *9*, e53404. https://doi.org/10.7554/eLife.53404

Münch, J., & Abdelilah-Seyfried, S. (2021). Sensing and Responding of Cardiomyocytes to Changes of Tissue Stiffness in the Diseased Heart. *Frontiers in Cell and Developmental Biology*, *9*, 642840. https://doi.org/10.3389/fcell.2021.642840

Namazi, H., Mohit, E., Namazi, I., Rajabi, S., Samadian, A., Hajizadeh-Saffar, E., Aghdami, N., & Baharvand, H. (2018). Exosomes secreted by hypoxic cardiosphere-derived cells enhance tube formation and increase pro-angiogenic miRNA. *Journal of Cellular Biochemistry*, *119*(5), 4150–4160. https://doi.org/10.1002/jcb.26621

Narang, P., Shah, M., & Beljanski, V. (2022). Exosomal RNAs in diagnosis and therapies. *Non-Coding RNA Research*, *7*(1), 7–15. https://doi.org/10.1016/j.ncrna.2022.01.001

Oeckinghaus, A., & Ghosh, S. (2009). The NF-κB Family of Transcription Factors and Its Regulation. *Cold Spring Harbor Perspectives in Biology*, *1*(4), a000034. https://doi.org/10.1101/cshperspect.a000034

O'Meara, C. C., Wamstad, J. A., Gladstone, R. A., Fomovsky, G. M., Butty, V. L., Shrikumar, A., Gannon, J. B., Boyer, L. A., & Lee, R. T. (2015). Transcriptional Reversion of Cardiac Myocyte Fate During Mammalian Cardiac Regeneration. *Circulation Research*, *116*(5), 804–815. https://doi.org/10.1161/CIRCRESAHA.116.304269

Ortuño-Costela, M. del C., Cerrada, V., García-López, M., & Gallardo, M. E. (2019). The Challenge of Bringing iPSCs to the Patient. *International Journal of Molecular Sciences*, *20*(24), 6305. https://doi.org/10.3390/ijms20246305

Ou, H., Teng, H., Qin, Y., Luo, X., Yang, P., Zhang, W., Chen, W., Lv, D., & Tang, H. (2020). Extracellular vesicles derived from microRNA-150-5p-overexpressing mesenchymal stem cells protect rat hearts against ischemia/reperfusion. *Aging (Albany NY)*, *12*(13), 12669–12683. https://doi.org/10.18632/aging.102792

Ozhan, G., & Weidinger, G. (2015). Wnt/β-catenin signaling in heart regeneration. *Cell Regeneration*, *4*, 3. https://doi.org/10.1186/s13619-015-0017-8

Park, E. J., Oh, S. Y. E.-J., Hong, S. H., & Park, C. G. K. and S. D. (1996). Simplified Electrophoresis Method for Analyses of High Molecular Weight RNA. *Molecules and Cells*, *6*(1), 23–26.

Park, H., Park, H., Mun, D., Kang, J., Kim, H., Kim, M., Cui, S., Lee, S.-H., & Joung, B. (2018). Extracellular Vesicles Derived from Hypoxic Human Mesenchymal Stem Cells Attenuate GSK3β Expression via miRNA-26a in an Ischemia-Reperfusion Injury Model. *Yonsei Medical Journal*, *59*(6), 736–745. https://doi.org/10.3349/ymj.2018.59.6.736

Pegtel, D. M., & Gould, S. J. (2019). Exosomes. *Annual Review of Biochemistry*, *88*(1), 487–514. https://doi.org/10.1146/annurev-biochem-013118-111902

Peng, Y., Zhao, J.-L., Peng, Z.-Y., Xu, W.-F., & Yu, G.-L. (2020). Exosomal miR-25-3p from mesenchymal stem cells alleviates myocardial infarction by targeting pro-apoptotic proteins and EZH2. *Cell Death & Disease*, *11*(5), 1–15. https://doi.org/10.1038/s41419-020-2545-6

Piccolo, S., Dupont, S., & Cordenonsi, M. (2014). The Biology of YAP/TAZ: Hippo Signaling and Beyond. *Physiological Reviews*, *94*(4), 1287–1312. https://doi.org/10.1152/physrev.00005.2014

Porrello, E. R., Mahmoud, A. I., Simpson, E., Hill, J. A., Richardson, J. A., Olson, E. N., & Sadek, H. A. (2011). Transient Regenerative Potential of the Neonatal Mouse Heart. *Science (New York, N.Y.)*, *331*(6020), 1078–1080. https://doi.org/10.1126/science.1200708

Porter Jr., G. A., Makuck, R. F., & Rivkees, S. A. (2003). Intracellular calcium plays an essential role in cardiac development. *Developmental Dynamics*, *227*(2), 280–290. https://doi.org/10.1002/dvdy.10307

Powers, N., & Huang, G. N. (2022). Mending a broken heart with novel cardiogenic small molecules. *Cell Regeneration*, *11*(1), 20. https://doi.org/10.1186/s13619-022-00120-z

Qiao, L., Hu, S., Liu, S., Zhang, H., Ma, H., Huang, K., Li, Z., Su, T., Vandergriff, A., Tang, J., Allen, T., Dinh, P.-U., Cores, J., Yin, Q., Li, Y., & Cheng, K. (2019). MicroRNA-21-5p dysregulation in exosomes derived from heart failure patients

impairs regenerative potential. *The Journal of Clinical Investigation*, *129*(6), 2237–2250. https://doi.org/10.1172/JCI123135

Rozengurt, E., Sinnett-Smith, J., & Eibl, G. (2018). Yes-associated protein (YAP) in pancreatic cancer: At the epicenter of a targetable signaling network associated with patient survival. *Signal Transduction and Targeted Therapy*, *3*(1), 1–10. https://doi.org/10.1038/s41392-017-0005-2

Santoni-Rugiu, E., Falck, J., Mailand, N., Bartek, J., & Lukas, J. (2000). Involvement of Myc Activity in a G1/S-Promoting Mechanism Parallel to the pRb/E2F Pathway. *Molecular and Cellular Biology*, *20*(10), 3497–3509.

Santoso, M. R., Ikeda, G., Tada, Y., Jung, J., Vaskova, E., Sierra, R. G., Gati, C., Goldstone, A. B., von Bornstaedt, D., Shukla, P., Wu, J. C., Wakatsuki, S., Woo, Y. J., & Yang, P. C. (2020). Exosomes From Induced Pluripotent Stem Cell–Derived Cardiomyocytes Promote Autophagy for Myocardial Repair. *Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease*, *9*(6), e014345. https://doi.org/10.1161/JAHA.119.014345

Savarese, G., Becher, P. M., Lund, L. H., Seferovic, P., Rosano, G. M. C., & Coats, A. J. S. (2022). Global burden of heart failure: A comprehensive and updated review of epidemiology. *Cardiovascular Research*, cvac013. https://doi.org/10.1093/cvr/cvac013

Saxton, R. A., & Sabatini, D. M. (2017). MTOR Signaling in Growth, Metabolism, and Disease. *Cell*, *168*(6), 960–976. https://doi.org/10.1016/j.cell.2017.02.004

Scalise, M., Marino, F., Salerno, L., Mancuso, T., Cappetta, D., Barone, A., Parrotta, E. I., Torella, A., Palumbo, D., Veltri, P., De Angelis, A., Berrino, L., Rossi, F., Weisz, A., Rota, M., Urbanek, K., Nadal-Ginard, B., Torella, D., & Cianflone, E. (2021). In vitro CSC-derived cardiomyocytes exhibit the typical microRNA-mRNA blueprint of endogenous cardiomyocytes. *Communications Biology*, *4*(1), 1–16. https://doi.org/10.1038/s42003-021-02677-y

Sciarretta, S., Forte, M., Frati, G., & Sadoshima, J. (2018). New insights into the role of mTOR signaling in the cardiovascular system. *Circulation Research*, *122*(3), 489–505. https://doi.org/10.1161/CIRCRESAHA.117.311147

Seo, J., & Kim, J. (2018). Regulation of Hippo signaling by actin remodeling. *BMB Reports*, *51*(3), 151–156. https://doi.org/10.5483/BMBRep.2018.51.3.012

Serrano-Pertierra, E., Oliveira-Rodríguez, M., Rivas, M., Oliva, P., Villafani, J., Navarro, A., Blanco-López, M. C., & Cernuda-Morollón, E. (2019). Characterization of Plasma-Derived Extracellular Vesicles Isolated by Different Methods: A Comparison Study. *Bioengineering*, *6*(1), 8. https://doi.org/10.3390/bioengineering6010008

Shao, L., Zhang, Y., Lan, B., Wang, J., Zhang, Z., Zhang, L., Xiao, P., Meng, Q., Geng, Y., Yu, X., & Li, Y. (2017). MiRNA-Sequence Indicates That Mesenchymal Stem Cells and Exosomes Have Similar Mechanism to Enhance Cardiac Repair. *BioMed Research International*, *2017*, 4150705. https://doi.org/10.1155/2017/4150705

Sharma, A., Zhang, Y., & Wu, S. M. (2015). Harnessing the Induction of Cardiomyocyte Proliferation for Cardiac Regenerative Medicine. *Current Treatment Options in Cardiovascular Medicine*, *17*(10), 45. https://doi.org/10.1007/s11936-015-0404-z

Shelke, G. V., Lässer, C., Gho, Y. S., & Lötvall, J. (2014). Importance of exosome depletion protocols to eliminate functional and RNA-containing extracellular vesicles from fetal bovine serum. *Journal of Extracellular Vesicles*, *3*, 10.3402/jev.v3.24783. https://doi.org/10.3402/jev.v3.24783

Shome, D., von Woedtke, T., Riedel, K., & Masur, K. (2020). The HIPPO Transducer YAP and Its Targets CTGF and Cyr61 Drive a Paracrine Signalling in Cold Atmospheric Plasma-Mediated Wound Healing. *Oxidative Medicine and Cellular Longevity*, *2020*, e4910280. https://doi.org/10.1155/2020/4910280

Shouman, S., Zaher, A., Abdelhameed, A., Elshaboury, S., Sakr, S., Fouda, B. E., Mohamed, H., & El-Badri, N. (2021). Cardiac Progenitor Cells. In K. Turksen (Ed.), *Cell Biology and Translational Medicine, Volume 11: Stem Cell Therapy—Potential and Challenges* (pp. 51–73). Springer International Publishing. https://doi.org/10.1007/5584_2020_594

Sidhom, K., Obi, P. O., & Saleem, A. (2020). A Review of Exosomal Isolation Methods: Is Size Exclusion Chromatography the Best Option? *International Journal of Molecular Sciences*, *21*(18), 6466. https://doi.org/10.3390/ijms21186466

Smith, R. R., Barile, L., Cho, H. C., Leppo, M. K., Hare, J. M., Messina, E., Giacomello, A., Abraham, M. R., & Marbán, E. (2007). Regenerative Potential of Cardiosphere-Derived Cells Expanded From Percutaneous Endomyocardial

Biopsy Specimens. *Circulation*, *115*(7), 896–908. https://doi.org/10.1161/CIRCULATIONAHA.106.655209

Song, H.-P., Chu, Z.-G., Zhang, D.-X., Dang, Y.-M., & Zhang, Q. (2018). PI3K–AKT Pathway Protects Cardiomyocytes Against Hypoxia-Induced Apoptosis by MitoKATP-Mediated Mitochondrial Translocation of pAKT. *Cellular Physiology and Biochemistry*, *49*(2), 717–727. https://doi.org/10.1159/000493037

Sun, J., Shen, H., Shao, L., Teng, X., Chen, Y., Liu, X., Yang, Z., & Shen, Z. (2020). HIF-1α overexpression in mesenchymal stem cell-derived exosomes mediates cardioprotection in myocardial infarction by enhanced angiogenesis. *Stem Cell Research & Therapy*, *11*(1), 373. https://doi.org/10.1186/s13287-020-01881-7

Sun, X., Sit, A., & Feinberg, M. W. (2014). Role for miR-181 family in regulating vascular inflammation and immunity. *Trends in Cardiovascular Medicine*, *24*(3), 105–112. https://doi.org/10.1016/j.tcm.2013.09.002

Sutton, M. G. St. J., & Sharpe, N. (2000). Left Ventricular Remodeling After Myocardial Infarction. *Circulation*, *101*(25), 2981–2988. https://doi.org/10.1161/01.CIR.101.25.2981

Tang, J., Shen, D., Caranasos, T. G., Wang, Z., Vandergriff, A. C., Allen, T. A., Hensley, M. T., Dinh, P.-U., Cores, J., Li, T.-S., Zhang, J., Kan, Q., & Cheng, K. (2017). Therapeutic microparticles functionalized with biomimetic cardiac stem cell membranes and secretome. *Nature Communications*, *8*, 13724. https://doi.org/10.1038/ncomms13724

Teng, F., & Fussenegger, M. (2021). Shedding Light on Extracellular Vesicle Biogenesis and Bioengineering. *Advanced Science*, *8*(1), 2003505. https://doi.org/10.1002/advs.202003505

Thej, C., & Kishore, R. (2021). Unfathomed Nanomessages to the Heart: Translational Implications of Stem Cell-Derived, Progenitor Cell Exosomes in Cardiac Repair and Regeneration. *Cells*, *10*(7), 1811. https://doi.org/10.3390/cells10071811

Théry, C., Amigorena, S., Raposo, G., & Clayton, A. (2006). Isolation and Characterization of Exosomes from Cell Culture Supernatants and Biological Fluids. *Current Protocols in Cell Biology*, *30*(1), 3.22.1-3.22.29. https://doi.org/10.1002/0471143030.cb0322s30

Théry, C., Witwer, K. W., Aikawa, E., Alcaraz, M. J., Anderson, J. D., Andriantsitohaina, R., Antoniou, A., Arab, T., Archer, F., Atkin-Smith, G. K., Ayre, D. C., Bach, J.-M., Bachurski, D., Baharvand, H., Balaj, L., Baldacchino, S., Bauer, N. N., Baxter, A. A., Bebawy, M., … Zuba-Surma, E. K. (2018). Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *Journal of Extracellular Vesicles*, *7*(1), 1535750. https://doi.org/10.1080/20013078.2018.1535750

Thomas, T. P., & Grisanti, L. A. (2020). The Dynamic Interplay Between Cardiac Inflammation and Fibrosis. *Frontiers in Physiology*, *11*. https://www.frontiersin.org/article/10.3389/fphys.2020.529075

Trummer, A., De Rop, C., Tiede, A., Ganser, A., & Eisert, R. (2009). Recovery and composition of microparticles after snap-freezing depends on thawing temperature. *Blood Coagulation & Fibrinolysis: An International Journal in Haemostasis and Thrombosis*, *20*(1), 52–56. https://doi.org/10.1097/MBC.0b013e32831be9c5

Urbanelli, L., Magini, A., Buratta, S., Brozzi, A., Sagini, K., Polchi, A., Tancini, B., & Emiliani, C. (2013). Signaling Pathways in Exosomes Biogenesis, Secretion and Fate. *Genes*, *4*(2), 152–170. https://doi.org/10.3390/genes4020152

Uzdensky, A. B., Demyanenko, S. V., & Bibov, M. Y. (2013). Signal transduction in human cutaneous melanoma and target drugs. *Current Cancer Drug Targets*, *13*(8), 843–866. https://doi.org/10.2174/1568009611313080004

Vandergriff, A., Huang, K., Shen, D., Hu, S., Hensley, M. T., Caranasos, T. G., Qian, L., & Cheng, K. (2018). Targeting regenerative exosomes to myocardial infarction using cardiac homing peptide. *Theranostics*, *8*(7), 1869–1878. https://doi.org/10.7150/thno.20524

Vasudevan, H. N., Mazot, P., He, F., & Soriano, P. (2015). Receptor tyrosine kinases modulate distinct transcriptional programs by differential usage of intracellular pathways. *ELife*, *4*, e07186. https://doi.org/10.7554/eLife.07186

Velayutham, N., Agnew, E. J., & Yutzey, K. E. (2019). Postnatal cardiac development and regenerative potential in large mammals. *Pediatric Cardiology*, *40*(7), 1345–1358. https://doi.org/10.1007/s00246-019-02163-7

Vlachos, I. S., Zagganas, K., Paraskevopoulou, M. D., Georgakilas, G., Karagkouni, D., Vergoulis, T., Dalamagas, T., & Hatzigeorgiou, A. G. (2015). DIANA-miRPath v3.0: Deciphering microRNA function with experimental support. *Nucleic Acids Research*, *43*(Web Server issue), W460–W466. https://doi.org/10.1093/nar/gkv403

Volarevic, V., Markovic, B. S., Gazdic, M., Volarevic, A., Jovicic, N., Arsenijevic, N., Armstrong, L., Djonov, V., Lako, M., & Stojkovic, M. (2018). Ethical and Safety Issues of Stem Cell-Based Therapy. *International Journal of Medical Sciences*, *15*(1), 36–45. https://doi.org/10.7150/ijms.21666

Vujic, A., Natarajan, N., & Lee, R. T. (2020). Molecular mechanisms of heart regeneration. *Seminars in Cell & Developmental Biology*, *100*, 20–28. https://doi.org/10.1016/j.semcdb.2019.09.003

Wadugu, B., & Kühn, B. (2012). The role of neuregulin/ErbB2/ErbB4 signaling in the heart with special focus on effects on cardiomyocyte proliferation. *American Journal of Physiology - Heart and Circulatory Physiology*, *302*(11), H2139–H2147. https://doi.org/10.1152/ajpheart.00063.2012

Wang, H., Xu, J., Lazarovici, P., Quirion, R., & Zheng, W. (2018). cAMP Response Element-Binding Protein (CREB): A Possible Signaling Molecule Link in the Pathophysiology of Schizophrenia. *Frontiers in Molecular Neuroscience*, *11*. https://www.frontiersin.org/article/10.3389/fnmol.2018.00255

Wang, J., Liu, M., Wu, Q., Li, Q., Gao, L., Jiang, Y., Deng, B., Huang, W., Bi, W., Chen, Z., Chin, Y. E., Paul, C., Wang, Y., & Yang, H.-T. (2019). Human Embryonic

Stem Cell-Derived Cardiovascular Progenitors Repair Infarcted Hearts Through Modulation of Macrophages via Activation of Signal Transducer and Activator of Transcription 6. *Antioxidants & Redox Signaling*, *31*(5), 369–386. https://doi.org/10.1089/ars.2018.7688

Wang, J., Liu, S., Heallen, T., & Martin, J. F. (2018). The Hippo pathway in the heart: Pivotal roles in development, disease, and regeneration. *Nature Reviews Cardiology*, *15*(11), 672–684. https://doi.org/10.1038/s41569-018-0063-3

Wang, J., Ma, L., Weng, W., Qiao, Y., Zhang, Y., He, J., Wang, H., Xiao, W., Li, L., Chu, Q., Pan, Q., Yu, Y., & Sun, F. (2013). Mutual interaction between YAP and CREB promotes tumorigenesis in liver cancer. *Hepatology (Baltimore, Md.)*, *58*(3), 1011–1020. https://doi.org/10.1002/hep.26420

Wang, L., Jia, Q., Xinnong, C., Xie, Y., Yang, Y., Zhang, A., Liu, R., Zhuo, Y., & Zhang, J. (2019). Role of cardiac progenitor cell-derived exosome-mediated microRNA-210 in cardiovascular disease. *Journal of Cellular and Molecular Medicine*, *23*(11), 7124–7131. https://doi.org/10.1111/jcmm.14562

Wang, Q., He, G., Hou, M., Chen, L., Chen, S., Xu, A., & Fu, Y. (2018). Cell Cycle Regulation by Alternative Polyadenylation of CCND1. *Scientific Reports*, *8*, 6824. https://doi.org/10.1038/s41598-018-25141-0

Wang, X., Chen, Y., Zhao, Z., Meng, Q., Yu, Y., Sun, J., Yang, Z., Chen, Y., Li, J., Ma, T., Liu, H., Li, Z., Yang, J., & Shen, Z. (2018). Engineered Exosomes With Ischemic Myocardium-Targeting Peptide for Targeted Therapy in Myocardial Infarction. *Journal of the American Heart Association: Cardiovascular and*

Cerebrovascular Disease, *7*(15), e008737. https://doi.org/10.1161/JAHA.118.008737

Wang, X., Morelli, M. B., Matarese, A., Sardu, C., & Santulli, G. (2020). Cardiomyocyte-derived exosomal microRNA-92a mediates post-ischemic myofibroblast activation both in vitro and ex vivo. *ESC Heart Failure*, *7*(1), 284–288. https://doi.org/10.1002/ehf2.12584

Weinberger, F., & Eschenhagen, T. (2021). Cardiac Regeneration: New Hope for an Old Dream. *Annual Review of Physiology*, *83*(1), 59–81. https://doi.org/10.1146/annurev-physiol-031120-103629

Wen, Z., Mai, Z., Zhu, X., Wu, T., Chen, Y., Geng, D., & Wang, J. (2020). Mesenchymal stem cell-derived exosomes ameliorate cardiomyocyte apoptosis in hypoxic conditions through microRNA144 by targeting the PTEN/AKT pathway. *Stem Cell Research & Therapy*, *11*(1), 36. https://doi.org/10.1186/s13287-020-1563-8

Witman, N., Zhou, C., Grote Beverborg, N., Sahara, M., & Chien, K. R. (2020). Cardiac progenitors and paracrine mediators in cardiogenesis and heart regeneration. *Seminars in Cell & Developmental Biology*, *100*, 29–51. https://doi.org/10.1016/j.semcdb.2019.10.011

Witwer, K. W., & Théry, C. (2019). Extracellular vesicles or exosomes? On primacy, precision, and popularity influencing a choice of nomenclature. *Journal of Extracellular Vesicles*, *8*(1), 1648167. https://doi.org/10.1080/20013078.2019.1648167

Wu, C., Gong, Y., Yuan, J., Zhang, W., Zhao, G., Li, H., Sun, A., Zou, Y., & Ge, J. (2012). MicroRNA-181a represses ox-LDL-stimulated inflammatory response in dendritic cell by targeting c-Fos. *Journal of Lipid Research*, *53*(11), 2355–2363. https://doi.org/10.1194/jlr.M028878

Wu, Q., Wang, J., Tan, W. L. W., Jiang, Y., Wang, S., Li, Q., Yu, X., Tan, J., Liu, S., Zhang, P., Tiang, Z., Chen, Z., Foo, R. S.-Y., & Yang, H.-T. (2020). Extracellular vesicles from human embryonic stem cell-derived cardiovascular progenitor cells promote cardiac infarct healing through reducing cardiomyocyte death and promoting angiogenesis. *Cell Death & Disease*, *11*(5), 1–16. https://doi.org/10.1038/s41419-020-2508-y

Wu, S. M., Fujiwara, Y., Cibulsky, S. M., Clapham, D. E., Lien, C., Schultheiss, T. M., & Orkin, S. H. (2006). Developmental Origin of a Bipotential Myocardial and Smooth Muscle Cell Precursor in the Mammalian Heart. *Cell*, *127*(6), 1137–1150. https://doi.org/10.1016/j.cell.2006.10.028

Xiao, J., Pan, Y., Li, X. H., Yang, X. Y., Feng, Y. L., Tan, H. H., Jiang, L., Feng, J., & Yu, X. Y. (2016). Cardiac progenitor cell-derived exosomes prevent cardiomyocytes apoptosis through exosomal miR-21 by targeting PDCD4. *Cell Death & Disease*, *7*(6), e2277. https://doi.org/10.1038/cddis.2016.181

Xin, M., Kim, Y., Sutherland, L. B., Murakami, M., Qi, X., McAnally, J., Porrello, E. R., Mahmoud, A. I., Tan, W., Shelton, J. M., Richardson, J. A., Sadek, H. A., Bassel-Duby, R., & Olson, E. N. (2013). Hippo pathway effector Yap promotes cardiac

regeneration. *Proceedings of the National Academy of Sciences*, *110*(34), 13839–13844. https://doi.org/10.1073/pnas.1313192110

Xing, Z., Wang, X., Liu, J., Zhang, M., Feng, K., & Wang, X. (2021). Expression and prognostic value of CDK1, CCNA2, and CCNB1 gene clusters in human breast cancer. *The Journal of International Medical Research*, *49*(4), 0300060520980647. https://doi.org/10.1177/0300060520980647

Xu, K., Jin, Y., Li, Y., Huang, Y., & Zhao, R. (2022). Recent Progress of Exosome Isolation and Peptide Recognition-Guided Strategies for Exosome Research. *Frontiers in Chemistry*, *10*. https://www.frontiersin.org/article/10.3389/fchem.2022.844124

Xuan, W., Khan, M., & Ashraf, M. (2020). Extracellular Vesicles From Notch Activated Cardiac Mesenchymal Stem Cells Promote Myocyte Proliferation and Neovasculogenesis. *Frontiers in Cell and Developmental Biology*, *8*. https://www.frontiersin.org/article/10.3389/fcell.2020.00011

Xue, G., & Hemmings, B. A. (2013). PKB/Akt–Dependent Regulation of Cell Motility. *JNCI: Journal of the National Cancer Institute*, *105*(6), 393–404. https://doi.org/10.1093/jnci/djs648

Yamaguchi, H., & Taouk, G. M. (2020). A Potential Role of YAP/TAZ in the Interplay Between Metastasis and Metabolic Alterations. *Frontiers in Oncology*, *10*. https://www.frontiersin.org/article/10.3389/fonc.2020.00928

Yamanaka, S., Li, J., Kania, G., Elliott, S., Wersto, R. P., Van Eyk, J., Wobus, A. M., & Boheler, K. R. (2008). Pluripotency of embryonic stem cells. *Cell and Tissue Research*, *331*(1), 5–22. https://doi.org/10.1007/s00441-007-0520-5

Yancy, C. W., Jessup, M., Bozkurt, B., Butler, J., Casey, D. E., Drazner, M. H., Fonarow, G. C., Geraci, S. A., Horwich, T., Januzzi, J. L., Johnson, M. R., Kasper, E. K., Levy, W. C., Masoudi, F. A., McBride, P. E., McMurray, J. J. V., Mitchell, J. E., Peterson, P. N., Riegel, B., … Wilkoff, B. L. (2013). 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Journal of the American College of Cardiology*, *62*(16), e147–e239. https://doi.org/10.1016/j.jacc.2013.05.019

Yao, J., Huang, K., Zhu, D., Chen, T., Jiang, Y., Zhang, J., Mi, L., Xuan, H., Hu, S., Li, J., Zhou, Y., & Cheng, K. (2021). A Minimally Invasive Exosome Spray Repairs Heart after Myocardial Infarction. *ACS Nano*, *15*(7), 11099–11111. https://doi.org/10.1021/acsnano.1c00628

Ye, L., D'Agostino, G., Loo, S. J., Wang, C. X., Su, L. P., Tan, S. H., Tee, G. Z., Pua, C. J., Pena, E. M., Cheng, R. B., Chen, W. C., Abdurrachim, D., Lalic, J., Tan, R. S., Lee, T. H., Zhang, J., & Cook, S. A. (2018). Early Regenerative Capacity in the Porcine Heart. *Circulation*, *138*(24), 2798–2808. https://doi.org/10.1161/CIRCULATIONAHA.117.031542

Yi, Y. W., Lee, J. H., Kim, S.-Y., Pack, C.-G., Ha, D. H., Park, S. R., Youn, J., & Cho, B. S. (2020). Advances in Analysis of Biodistribution of Exosomes by Molecular

Imaging. *International Journal of Molecular Sciences*, *21*(2), 665. https://doi.org/10.3390/ijms21020665

Yu, Q., Li, W., Jin, R., Yu, S., Xie, D., Zheng, X., Zhong, W., Cheng, X., Hu, S., Li, M., Zheng, Q., Li, G., & Song, Z. (2019). PI3Kγ (Phosphoinositide 3-Kinase γ) Regulates Vascular Smooth Muscle Cell Phenotypic Modulation and Neointimal Formation Through CREB (Cyclic AMP-Response Element Binding Protein)/YAP (Yes-Associated Protein) Signaling. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *39*(3), e91–e105. https://doi.org/10.1161/ATVBAHA.118.312212

Yue, R., Lu, S., Luo, Y., Zeng, J., Liang, H., Qin, D., Wang, X., Wang, T., Pu, J., & Hu, H. (2022). Mesenchymal stem cell-derived exosomal microRNA-182-5p alleviates myocardial ischemia/reperfusion injury by targeting GSDMD in mice. *Cell Death Discovery*, *8*(1), 1–14. https://doi.org/10.1038/s41420-022-00909-6

Zanconato, F., Forcato, M., Battilana, G., Azzolin, L., Quaranta, E., Bodega, B., Rosato, A., Bicciato, S., Cordenonsi, M., & Piccolo, S. (2015). Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. *Nature Cell Biology*, *17*(9), 1218–1227. https://doi.org/10.1038/ncb3216

Zhang, J., Bolli, R., Garry, D. J., Marbán, E., Menasché, P., Zimmermann, W.-H., Kamp, T. J., Wu, J. C., & Dzau, V. J. (2021). Basic and Translational Research in Cardiac Repair and Regeneration. *Journal of the American College of Cardiology*, *78*(21), 2092–2105. https://doi.org/10.1016/j.jacc.2021.09.019

Zhang, K., Wong, P., Zhang, L., Jacobs, B., Borden, E. C., Aster, J. C., & Bedogni, B. (2012). A Notch1-neuregulin1 autocrine signaling loop contributes to melanoma growth. *Oncogene*, *31*(43), 4609–4618. https://doi.org/10.1038/onc.2011.606

Zhang, Y., Bi, J., Huang, J., Tang, Y., Du, S., & Li, P. (2020). Exosome: A Review of Its Classification, Isolation Techniques, Storage, Diagnostic and Targeted Therapy Applications. *International Journal of Nanomedicine*, *15*, 6917–6934. https://doi.org/10.2147/IJN.S264498

Zhao, B., Ye, X., Yu, J., Li, L., Li, W., Li, S., Yu, J., Lin, J. D., Wang, C.-Y., Chinnaiyan, A. M., Lai, Z.-C., & Guan, K.-L. (2008). TEAD mediates YAP-dependent gene induction and growth control. *Genes & Development*, *22*(14), 1962–1971. https://doi.org/10.1101/gad.1664408

Zhao, J., Li, X., Hu, J., Chen, F., Qiao, S., Sun, X., Gao, L., Xie, J., & Xu, B. (2019). Mesenchymal stromal cell-derived exosomes attenuate myocardial ischaemia-reperfusion injury through miR-182-regulated macrophage polarization. *Cardiovascular Research*, *115*(7), 1205–1216. https://doi.org/10.1093/cvr/cvz040

Zhao, T., Wang, Z., Fang, J., Cheng, W., Zhang, Y., Huang, J., Xu, L., Gou, H., Zeng, L., Jin, Z., & Matsuoka, M. (2022). HTLV-1 activates YAP via NF-κB/p65 to promote oncogenesis. *Proceedings of the National Academy of Sciences*, *119*(9), e2115316119. https://doi.org/10.1073/pnas.2115316119

Zhou, Q., Li, L., Zhao, B., & Guan, K.-L. (2015). The Hippo pathway in heart development, regeneration, and diseases. *Circulation Research*, *116*(8), 1431–1447. https://doi.org/10.1161/CIRCRESAHA.116.303311

Zhu, D., & Cheng, K. (2021). Cardiac Cell Therapy for Heart Repair: Should the Cells Be Left Out? *Cells*, *10*(3), 641. https://doi.org/10.3390/cells10030641

Zhu, D., Wang, Y., Thomas, M., McLaughlin, K., Oguljahan, B., Henderson, J., Yang, Q., Chen, Y. E., & Liu, D. (2022). Exosomes from adipose-derived stem cells alleviate myocardial infarction via microRNA-31/FIH1/HIF-1α pathway. *Journal of Molecular and Cellular Cardiology*, *162*, 10–19. https://doi.org/10.1016/j.yjmcc.2021.08.010

Zhu, F., Meng, Q., Yu, Y., Shao, L., & Shen, Z. (2021). Adult Cardiomyocyte Proliferation: A New Insight for Myocardial Infarction Therapy. *Journal of Cardiovascular Translational Research*, *14*(3), 457–466. https://doi.org/10.1007/s12265-020-10067-8

Zhu, L.-P., Tian, T., Wang, J.-Y., He, J.-N., Chen, T., Pan, M., Xu, L., Zhang, H., Qiu, X.-T., Li, C.-C., Wang, K.-K., Shen, H., Zhang, G.-G., & Bai, Y.-P. (2018). Hypoxia-elicited mesenchymal stem cell-derived exosomes facilitates cardiac repair through miR-125b-mediated prevention of cell death in myocardial infarction. *Theranostics*, *8*(22), 6163–6177. https://doi.org/10.7150/thno.28021

Zhu, W., Zhang, E., Zhao, M., Chong, Z., Fan, C., Tang, Y., Hunter, J. D., Borovjagin, A. V., Walcott, G. P., Chen, J. Y., Qin, G., & Zhang, J. (2018). Regenerative Potential of Neonatal Porcine Hearts. *Circulation*, *138*(24), 2809–2816. https://doi.org/10.1161/CIRCULATIONAHA.118.034886

Zhu, Y., Do, V. D., Richards, A. M., & Foo, R. (2021). What we know about cardiomyocyte dedifferentiation. *Journal of Molecular and Cellular Cardiology*, *152*, 80–91. https://doi.org/10.1016/j.yjmcc.2020.11.016