

Cell therapy-induced recovery of dysfunctional microvasculature

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In aging, circulating catecholamines rise to counter a decline in cardiac output. While initially protective, this can lead to decreased sensitivity of dilative microvascular beta-adrenergic receptors (β AR), increasing risk of ischemia through poor perfusion. Our lab has shown an intravenous injection of adipose stromal vascular fraction (SVF) significantly improves β 1AR mediated coronary flow reserve (CFR) and restores the vasodilatory capacity of coronary vessels to β 1AR agonists in aged female rats [1]. Our recent study in the journal *Geroscience* examined alterations in the signaling pathway of the β 1AR in response to aging that induced desensitization and are improved or unchanged due to SVF therapy [2]. The objective of this editorial is to address additional points of consideration and suggest new avenues worth investigating.

In aging female rats, there was an increase in plasma norepinephrine (NE) concentration that was reduced following SVF therapy (albeit not significant) [1]. However, when measuring urine catecholamines SVF did not reduce the significantly increased level of NE present in aging [2]. There was a significant reduction in transcription of catecholamine degradation enzyme catechol-o-methyltransferase (COMT, also degrades estrogens) following SVF therapy compared to aged-controls [2]. Importantly, aged-controls had both high NE concentration and higher (relative to SVF) COMT expression, while SVF-injected rats only exhibited high NE concentration (relative to aging), suggesting perhaps that untreated aged-controls are producing enough NE to overcome NE degradation by the enhanced presence of degradation enzymes. This data could indicate less total catecholamine production after SVF therapy which could have an impact on β 1AR functionality and desensitization.

Transcription of the β 1AR is not varied in aging or after SVF therapy (albeit β 2AR transcription was increased by SVF vs. aging) [2]. Therefore, we investigated the downstream signaling cascade and regulation of the β 1AR. GRK2 phosphorylates the β 1AR following catecholamine binding; an initial step in receptor internalization. While protein expression of GRK2 was unchanged between groups, there was an aging-associated increase in naturally active GRK2 compared to phosphorylated, naturally inactive, GRK2 (partially reversed by SVF). We showed that inhibition of GRK2's kinase activity improved β 1AR vasodilatory

sensitivity in coronary microvessels of aged and SVF-treated rats.

It's important to examine the contribution of nitric oxide (NO), considering its depletion in aging and its ability to inhibit GRK2-mediated β 1AR internalization. Since reactive oxygen species (ROS) can scavenge NO, we also examined the ROS-dependent effects on β 1AR desensitization and internalization. Coronary microvessels from aged rats were shown to have increased ROS and reduced bioavailability of NO [3]. Exogenous ROS incubation impairs young control β 1AR mediated dilation (mimicking aging) while exogenous low-dose sodium nitroprusside (NO donor) restores old control β 1AR mediated dilation (mimicking youth) [4]. Plentiful NO (such as during youth) naturally inhibits GRK2s by nitrosative post-translational modification. With SVF therapy, although NO bioavailability is not restored, ROS levels are reduced possibly alleviating β 1AR desensitization and internalization [3, 4].

SVF therapy may promote less impeded recycling with reduced degradation relative to aging. GRK2 recruits beta-arrestins to form clathrin/dynamin coated vesicles for receptor endocytosis into endosomes. PP2A dephosphorylates the β 1AR allowing for recycling back to the plasma membrane. Alpha-arrestin is responsible for ubiquitination-mediated internalization of β ARs as well as to delay their recycling [5, 6]. Of relevance, SVF significantly decreased alpha-arrestin 3 transcription compared to aged controls in isolated coronary microvessels [2].

SVF therapy also increased transcription of the estrogen related receptor- α and G-protein coupled estrogen receptor 1 (Gper1) compared to old control. Estrogen induces vasorelaxation through NO-dependent mechanisms in response to direct agonism or flow, and estrogen receptor alpha activation attenuates oxidative stress [7]. Additionally, estrogen and estrogen receptor- β are functionally linked to β AR receptor transcription and dilative response to norepinephrine [8]. Females of advanced age exhibit a drop in circulating estrogen levels, impairing microvascular function. Elevated estrogen receptor levels in SVF-treated rats may make their microvessels more sensitive to the limited circulating estrogen in aging, which could have regenerative effects in the context of enhanced β 1AR function after SVF-therapy.

Taken together our data suggests that aging results in β 1AR dysfunction through catecholamine overdrive, enhanced active (dephosphorylated) GRK2, accumulation of ROS, and loss of NO-mediated inhibition of GRK2 nitrosylation, all of which culminate in internalization of the β 1AR. Possible explanations for SVF-mediated recovery of coronary microvascular β 1AR vasodilatory capacity could be through a more homeostatic catecholamine microenvironment, reducing ROS accumulation and its mediation of desensitization and internalization, and possibly by improving β 1AR receptor recycling or reducing ubiquitination by reducing alpha arrestin 3 expression.

Future study is required to fully elucidate the mechanisms by which aging and SVF therapy modulate

β 1AR desensitization, internalization, and recycling (Figure 1). This is especially poignant in the context of regulatory post-translational modifications (thiol oxidation, nitrosylation, ubiquitination, phosphorylation) in part through ROS and NO, which fluctuate with aging and SVF therapy [3]. Protein expression and functional significance of alpha arrestin 3 on recycling and ubiquitination, as well as the significance of estrogen receptor alpha and Gper1 in the perseverance of β 1AR function are especially pertinent and require further investigation. Understanding the intricate mechanistic underpinnings of coronary microvascular dysfunction is of great importance considering first line therapies include beta blockers, which could potentiate the root cause of pathology despite providing symptomatic management.

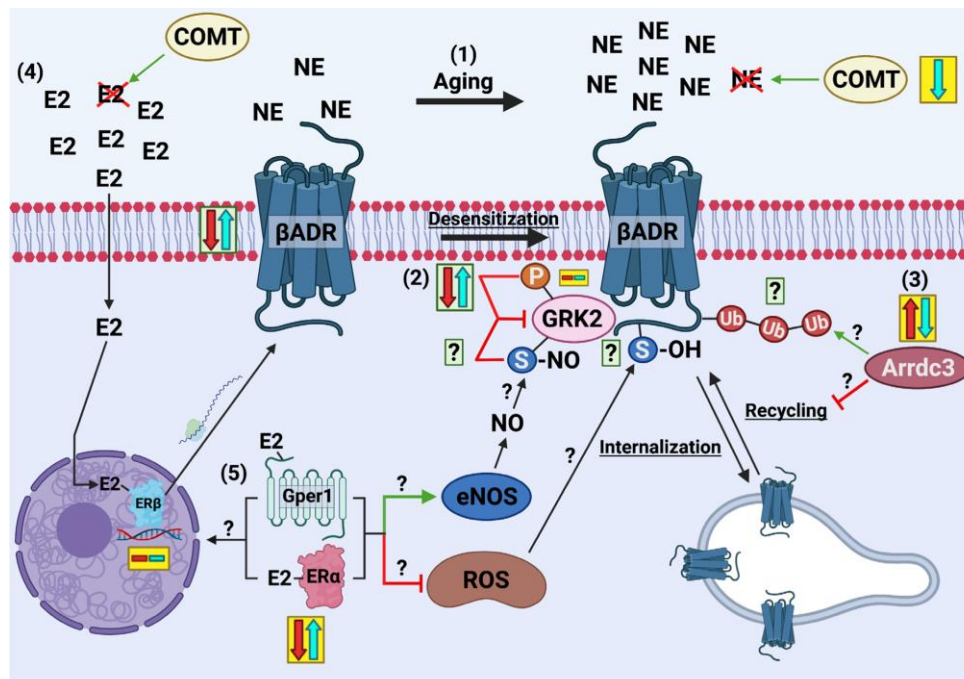


Figure 1. Potential contributing mechanisms of SVF-induced reversal of aging-mediated β ADR dysfunction.

In aging, there is an overdrive of catecholamine production that facilitates β ADR desensitization and internalization mediated by GRK2 (1). Transcription of catecholamine degradation enzyme COMT is significantly reduced by SVF therapy. There is no change in aging or SVF therapy in GRK2 transcription, however, naturally inhibited GRK2 (phosphorylated) is decreased in aging partially restored by SVF (albeit non-significantly) (2). Whether other post-translational modifications such as inhibitory nitric oxide-mediated S-nitrosylation of GRK2 contribute to SVF-mediated recovery of β ADR function warrants investigation. The transcription of alpha arrestin 3 (*arrdc3*) was significantly enhanced in aging and reversed by SVF (3). Whether this contributes to coronary microvascular β ADR dysfunction in aging, as it does in other vascular settings through β ADR ubiquitination and delayed recycling, remains unknown. In other vascular settings, estrogen enhances β ADR vasodilatory function and transcription via estrogen receptor- β , although there were no transcriptional differences between groups in our study (4). Transcription of estrogen receptor- α and Gper1 were significantly enhanced with SVF therapy, which may influence β ADR transcription or enhance nitric oxide or attenuate ROS production as they are known to do in other vascular settings, which could influence β ADR dilatory function (5). Yellow boxes represent gene expression whereas green boxes represent protein expression with red arrow indicating aging and blue arrow indicating SVF therapy. Question marks represent future directions to elucidate SVF-mediated recovery of β ADR function based on RNA sequencing data. Image created with BioRender.com.

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