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Editorial commentary: Epithelial-to-Mesenchymal transition and G protein-coupled receptors: A novel possibility for cardiac regeneration?

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Cardiovascular diseases (CVDs) remain a major cause of morbidity and mortality worldwide. Various metabolic disorders like obesity, diabetes and high blood pressure sequentially cause numerous CVDs like hypertension, cardiomyopathies, cardiac arrest, cardiac valve calcification and arrhythmias among others [1]. The swelling numbers of global deaths constantly craft a call for effective Epithelial-to-Mesenchymal Transition tools that can possibly alleviate CVDs, shaping current stream of cardiovascular research into exemplified inter-systemic approaches aimed for generating therapeutic strategies. Congenital heart diseases (CHDs) account for a high proportion of cardiac morbidity and mortality. Most common CHDs are defects in cardiac walls, valves and vessels carrying blood to and from the heart. Some of these such as small septal defects can be mild and need no treatment after birth, whereas, severe defects like hypoplastic left heart syndrome are lethal and need to be treated in time, triggering a need for understanding the mechanism of their occurrence and prognosis. With our increased understanding in cardiac structure, function, and development and early diagnosis, the numbers of children surviving after birth defects in the heart continue to increase [2].

Epithelial-to-Mesenchymal Transition (EMT) and Mesenchymal-to-Epithelial Transition (MET) are major contributing pathways in heart development. EMT and MET are involved in differentiation of animal cells into various tissues and organs, plays central role in organ development and assembly, including the heart. The embryonic development comprises several repetitions of EMT and MET resulting in formation of three-dimensional structures of mammalian organs, as reviewed in Thiery et al. [3]. Generally, three major sequential EMT repetitions (primary, secondary and tertiary) form a heart. Primary EMT is associated with early gastrulation steps generating cardiac progenitor cells. Secondary EMT further defines differentiation into several cardiac cell types and leads to formation of four compartments which result in cardiac primordium. It is the tertiary EMT that truly develops the heart primordia into the heart, when endothelial cells invade cardiac jelly forming endocardial cushion, terminally developing into the atrioventricular and valvulo-septal complex defining compartmentalization of the heart [4]. Fischer et al. [5] demonstrated that the transcription factors *Hey1* and *HeyL*, which co-operate during EMT lead into ventricular septal and atrioventricular pulmonary valve defects after their combined inactivation, while inactivation of *Hey2* leads to major congenital heart defects. The ErbB3 signaling pathway, which plays a fundamental role in EMT of endocardial cells resulting in atrio-ventricular canal, has a governing Gata4 transcription factor upstream. Heterozygous mutations in Gata4 have also been linked with congenital heart defects in humans [6].

G protein coupled receptors (GPCRs) are highly associated with cardiac development and concomitant congenital defects [7]. They are present in membranes of major cardiac cell types such as cardiomyocytes, fibroblasts and endothelial cells. They form a family of the most notably studied GPCRs such as cardiac function controlling β-adrenergic receptors and cardiac structure controlling angiotensin II, transducing extracellular signals into cells affecting several pathways. Of note, they are targets of almost 30% of currently available cardiovascular drugs [8].

In this issue of Trends in Cardiovascular Medicine, Nebigil and Desaubry [9] link GPCRs with the regulation of EMT during heart development. In addition, the authors also describe a promising option to redirect EMT after myocardial infarction for heart regeneration. Based on the sequence homology of GPCRs, these largest numbers of signaling molecules have been divided into six groups (A-F), namely rhodopsin-like receptors, secretin receptors, glutamate receptors, pheromone receptors, cAMP receptors and frizzled receptors [10]. Most notable ones in cardiac physiology are catecholaminergic members of group A, which control cardiovascular function by allocating actions of sympathetic nervous system. β-adrenergic and angiotensin II receptor blockers are the most crucial drugs involving GPCRs, counteracting adverse effects after chronic stimulation to induce cardioprotection. In addition to catecholamines, Nebigil and Desaubry [9] shed light on developmental and disease associated GPCRs like prokineticin receptor-1 (PKR1), sphingosine-1-phospate 1 receptor ($S1P_1R$) and serotonin (5HT)−2B receptors; belonging to prokineticin, sphingosine-1-phospate and serotonin receptor classes, respectively.

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Prokinectins are hormones that mediate cardiac development through proliferation and differentiation of epicardial cells via EMT. This finding was confirmed via conditional deletion of the PKR1 receptor, which caused embryonic lethality due to impaired EMT. In turn, its overexpression induced EMT-based remodeling as evidenced by upregulation of EMT-related gene expression profiles. PKR1 activation has also been found to improve epicardial cell morphology and actin cytoskeleton remodeling [11]. Importantly, Nebigil and Desaubry [9] also highlight the controlling of EMT by the PKR1 receptor to restrict formation of epicardial adipose tissues through epigenetic regulation involving demethylation of H3K27me3 and thus leading induction of endothelial and smooth muscle cells instead [12]. This strongly suggests a role of PKR1 receptor signaling in regulating epicardial EMT through epigenetic control and thus appears to be a viable target for cardiac tissue engineering.

Sphingosine-1-phospate receptors, consisting of five subtypes $S1P_1R$ - $S1P_5R$, have been associated with various cellular functions like differentiation, proliferation, migration, and apoptosis [13]. Embryonic lethality was observed in knockout mice of $S1P_1R$ due to defects in development of atrioventricular canal cushion, vascular maturation and myocardium [14]. This severe heart condition has been connected with altered EMT, which further disrupted fibronectin in epicardium, evidenced by disorganized myocardium in $S1P_1R^{-/-}$ hearts [15].

Serotonin receptors (5HT) are another GPCR family extensively studied for their regulatory functions in both the embryonic and adult heart. Among the 15 receptors subdivided in four classes, the serotonin 2B receptor is the most notable one, contributing in developmental and disease condition in heart. Serotonin 2B receptor deficient-mice displayed restricted embryonic lethality at midgestation, with the survived newborns suffering higher dilation due to considerably less number of cells and lower cell size. On other hand, overexpression of serotonin 2B receptors caused cardiac hypertrophy in transgenic mice with defects in mitochondria due to upregulation of ANT-1 and Bax [16]. In a different study, selective serotonin reuptake inhibitor (SSRI) Fluoxetine lead to dilated cardiomyopathy in rodents and cardiac malfunctions in humans, putting question mark over prenatal SSRI treatment [17].

Along these lines, the deletion of the Apelin receptor AgtrI1b causes embryonic lethality via growth retardation and heart malfunction. In addition, it promotes cardiac regeneration through endoderm regeneration and angiogenesis after treatment with ligand based hormonal drug Elabela [18]. Also, some differentially regulated GPCRs like GPR37L1 and GPR35 in cardiovascular diseases and myocardial infarction were identified, but their relation with EMT and disease treatment has not been established yet.

With this comprehensive and well-targeted review linking EMT/MET with GPCRs, the authors have established the notion, that EMT - as an important player in embryonic cardiac development and disease conditions - can be targeted via GPCR signaling resulting in the stimulation of favorable cardiac regeneration. This potential therapeutic strategy is well-supported by the possibility of activating *Hey* transcription factors or by clinical evidences of treatments through the example like Elabela. However before considering this strategy for therapeutic remedies, it is a primary need to establish the regulatory switch that arrests pathological pathway of EMT. Furthermore, being relatively hydrophobic membrane proteins, GPCRs remain highly difficult to crystalize and study, prompting an action mostly through ligand-based agonists or antagonists. Also, introduction of therapeutic agents at embryonic stage to induce EMT-based growth to avoid congenital defects itself is a challenging task. Except for PKR1 controlling EMT through epigenetic regulation, no other direct evidence has been established between EMT and GPCR signaling. Although the possibility of $S1P_1R$ playing key role in embryonic development via alteration in EMT is evident, further research in the context of epicardial growth control needs to be conducted before considering sphingosine 1-phosphate receptors for therapeutic strategy. Similarly, additional studies need to be conducted regarding SSRIs and thus serotonin receptors to employ them for clinical use, as they might impose threats like dilated cardiomyopathy in newborns or even abortion after prenatal SSRI treatment.

In conclusion, this review emphasizes the very central mechanism of embryonic and disease growth modulation through GPCRs, which can have tremendous clinical potential in cardiovascular disease managements. And thus, well-targeted research needs to be conducted to separate the thin line between clinical strategies and adverse pathological outcomes.

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References

[1] Wang Q, Shen JJ. Childhood Health Status and Adulthood Cardiovascular Disease Morbidity in Rural China: Are They Related? Int J Env Res Public Health 2016 Jun 6;13(6).
[2] Calcagni G, Unolt M, Digilio MC, Baban A, Versacci P, Tartaglia M, et al. Congenital heart disease and genetic syndromes: new insights into molecular mechanisms. Expert Rev Mol Diagn 2017 Sep;17(9):861–870.
[3] Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009;139(5):871–90.
[4] Nakajima Y, Yamagishi T, Hokari S, Nakamura H. Mechanisms involved in valvuloseptal endocardial cushion formation in early cardiogenesis: roles of transforming growth factor (TGF)-beta and bone morphogenetic protein (BMP). Anat Rec 2000;258(2):119–27.
[5] Fischer A, Steidl C, Wagner TU, Lang E, Jakob PM, Friedl P, et al. Combined loss of Hey1 and HeyL causes congenital heart defects because of impaired epithelial to mesenchymal transition. Circ Res 2007;100(6):856–63.
[6] Rivera-Feliciano J, Lee KH, Kong SW, Rajagopal S, Ma Q, Springer Z, et al. Development of heart valves requires Gata4 expression in endothelial-derived cells. Development 2006;133(18):3607–18.
[7] Kaye DM, Krum H. Drug discovery for heart failure: a new era or the end of the pipeline? Nat Rev Drug Discov 2007;6(2):127–39.
[8] Capote LA, Mendez Perez R, Lymperopoulos A. GPCR signaling and cardiac function. Eur J Pharmacol 2015;763(Pt B):143–8.
[9] Nebigil CG, Desaubry L. The role of GPCR signaling in cardiac epithelial to mesenchymal transformation (EMT). Trends Cardiovasc Med., 2018, pii: S1050-1738(18)30170-1.
[10] Gurevich VV, Gurevich EV. GPCRs and signal transducers: interaction stoichiometry. Trends Pharmacol Sci 2018;39(7):672–84.
[11] Arora H, Boulberdaa M, Qureshi R, Bitirim V, Gasser A, Messaddeq N, et al. Prokineticin receptor-1 signaling promotes epicardial to mesenchymal transition during heart development. Sci Rep 2016;6:25541.
[12] Qureshi R, Kindo M, Boulberdaa M, von Hunolstein JJ, Steenman M, Nebigil CG. A prokineticin-driven epigenetic switch regulates human epicardial cell stemness and fate. Stem Cells 2018.
[13] Mendelson K, Evans T, Hla T. Sphingosine 1-phosphate signalling. Development 2014;141(1):5–9.
[14] Liu YH, Yang XP, Mehta D, Bulagannawar M, Scicli GM, Carretero OA. Role of kinins in chronic heart failure and in the therapeutic effect of ACE inhibitors in kininogen-deficient rats. Am J Physiol Heart Circ Physiol 2000;278(2):H507–14.
[15] Kono Y, Nishiuma T, Nishimura Y, Kotani Y, Okada T, Nakamura S, et al. Sphingosine kinase 1 regulates differentiation of human and mouse lung fibroblasts mediated by TGF-beta1. Am J Respir Cell Mol. Biol 2007;37(4):395–404.
[16] Nebigil CG, Maroteaux L. Functional consequence of serotonin/5-HT2B receptor signaling in heart: role of mitochondria in transition between hypertrophy and heart failure? Circulation 2003;108(7):902–8.
[17] Cuzell JZ. Peroxetine may increase risk for congenital malformations. Dermatol Nurs 2006 Feb;18(1):68.
[18] Wang Z, Yu DZ, Wang MQ, Wang QL, Kouznetsova J, Yang RZ, et al. Elabela-apelin receptor signaling pathway is functional in mammalian systems. Sci Rep UK 2015 5.